We offer

Plant Pathol

for FALL TES

on Wheat, Oats and Barley

DU PONT

Dust Disinfectant

an effective Copper Compound for Certain

A TRIED, effective and inexpensive copp
has been used for two years on our ex
trolling stinking smut of wheat with s
Dust Disinfectant No. 63 in
dot smuts from about 20
Du Pont Dust Disinfectant No.
rate of two to three ounces per bushel of
per bushel of oats. The
reaction. Hydrogen, however, after reacting
with a small percentage
soluble than Copper Carbonate,
tests indicate Du Pont Dust Disinfect
effective than Copper Carbonate.

Send now for

E. I. DU PONT DE NEMOURS CO
Dyestuffs Department, Sales Di
Wilmington, Delaware

PHYTOPATHOLOGY

VOLUME 17 NUMBER 9

SEPTEMBER, 1927

A STUDY OF GROWTH HABIT AND RUST REACTION IN CROSSES BETWEEN MARQUIS, KOTA, AND KANRED WHEATS[1]

OLAF S. AAMODT[2]

INTRODUCTION

Information regarding the mode of inheritance of certain plant characters is to the plant breeder what good and useful tools are to the skilled mechanic. The more complicated and intricate the problem may appear, the greater is the need for fundamental knowledge of all phases of the problem. The mode of attack may possibly be altered considerably if one has available all of the facts concerning the mode of inheritance of a character, or characters. Crop-improvement programs are vitally concerned with such information. Naturally, then, it is desirable to gather as much information as possible on the genetics of our most important crop plants. The purpose of the present study was to ascertain the mode of inheritance of certain plant characters in wheat in relation to the problem of producing improved varieties of rust-resistant spring wheats.

HISTORICAL REVIEW

Growth Habit

Several previous studies of growth habit have been made, but an exact knowledge of the mode of inheritance of this character is not available. Spillman (33), in 1909, reported that the winter type was dominant over the spring type. Fruwirth (10) cites a report of Tschermak, who stated

[1] The data presented in this paper were obtained in cooperative investigations by the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Department of Agriculture of the University of Minnesota; and submitted to the graduate faculty of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy, granted June 13, 1927.

[2] The writer is indebted to Dr. H. K. Hayes, Professor of Plant Breeding, University of Minnesota, for valuable suggestions regarding the methods of conducting the study and in the interpretation of the results; and to Dr. E. C. Stakman, Professor of Plant Pathology, University of Minnesota, for suggestions on those phases of the investigation dealing with disease resistance.

that the F_1 plants from a cross between spring and winter wheats overwintered somewhat better than the winter parent and remained dormant throughout most of the summer, when the seed was spring-sown.

Caporn (5), in 1918, mentions that the F_1 from a cross between Polish (spring) and Rivett (winter) was intermediate for time of ripening. An "extensive scatter took place" in the F_2, and the bulk of the plants were earlier than the late parent.

Vavilov and Kuznetsova (37) appear to be the first to report a clear dominance of the spring character over the winter character in crosses between spring and winter wheats. They found a complicated segregation in the F_2 which was apparently due to the action of several factors. Several of the segregates were homozygous for different periods of heading in the F_3. The reported ratio in the F_2 was 9.6 early or late spring heading to 1 typical winter or non-heading.

The writer (1), in 1923, found the mode of inheritance of growth habit in a spring-winter wheat cross to be practically identical with that reported by Vavilov and Kuznetsova. There was a dominance of the spring type in F_1, a complicated segregation in F_2, and a number of lines in F_3 which were homozygous for various intermediate heading periods, as well as lines which bred true for winter and spring habit. Various types of segregation were observed in F_3.

Cooper (9) also studied crosses between spring and winter wheats. He found that the F_2 from some of the crosses segregated in the proportion of 13 springs to 3 winters, and in others the ratio was 3 springs to 1 winter. The 13:3 ratio was explained on the assumption that there was present a dominant factor for winter habit and an inhibitor factor of the winter habit which also was dominant. Twenty-three families were grown in the F_3 and not all of these were readily explainable on the same basis as the F_2 hypothesis.

Gaines and Singleton (13) obtained a ratio of 35 spring to 1 winter in the F_2 from a cross between a spring and a winter wheat. Their conclusions from this study are in agreement with those of Vavilov and Kuznetsova, and the earlier report by the writer. There is a dominance of the spring habit over the winter habit, and the inheritance of this character is controlled by multiple factors.

Rust Reaction

Complete reviews of literature have been made in previous publications on the breeding of wheats for resistance to physiologic forms of black stem rust. The present status of the problem and a statement of the mode of attack may be found in the papers published by Hayes and Stakman (19) and Hayes, Stakman and Aamodt (20). As the present paper is a report

on only one phase of the more extensive project of producing rust-resistant strains of common wheat, the literature review will be limited to those studies previously reported which have a direct bearing upon the particular aspects of the problem under consideration.

There are 37 physiologic forms of black stem rust (34), 21 of which have been found in the upper Mississippi Valley. It is desirable to obtain new varieties of common wheat which are resistant to all of these forms in the field. By certain crosses and re-crosses, it was thought such wheats could be produced synthetically. Information regarding certain theoretical considerations on the genetic relationship between the host and the pathogen would be of value in planning an intelligent mode of attack on the problem. Considerable evidence has been obtained to support the belief that a combination of the desirable characteristics of several wheat varieties can be obtained in a single variety. On several occasions (30, 14, 15) it was demonstrated, both in intraspecific and interspecific crosses, that the resistant reaction of two varieties of wheat to different physiologic forms could be combined in a single hybrid variety. Evidence has been obtained also that there may be one or several factor pairs in the host which govern the reaction to a single physiologic form (14, 15, 18, 19, 20, 30). In an earlier report (1), the writer demonstrated that one factor pair governed the reaction to several forms of stem rust in crosses between Marquis, a spring wheat, and Kanred, a winter wheat. It was also shown that this same factor for immunity from rust infection was inherited independently of the factors for spring and winter habit of growth in the two parents.

Immunity is complete resistance. It can be differentiated in the seedling stage in the greenhouse with the expectation that the strains probably also will be resistant in the field. The Kanred type of immunity from certain forms is of considerable importance in the breeding work because through its use immunity from eleven physiologic forms can be obtained. The fact that the same factor governs the reaction to all eleven forms simplifies the routine of determining whether a hybrid is immune from or susceptible to all of these forms. Hybrids from crosses in which this factor is concerned can be inoculated with one of the eleven forms from which Kanred is immune, and from the resulting reaction one may conclude that the hybrid is likewise immune from the other ten forms which are governed by this same factor. Simply by inoculating the F_3 seedlings in the greenhouse, during the winter, with a form of rust from which Kanred is immune, those segregates which are not homozygous for immunity from all eleven forms can be eliminated. The bulk of the material is thus considerably reduced and this facilitates the study of the reaction to other forms either in the greenhouse or field. Hayes, Stakman, and Aamodt (20) demonstrated that this factor for immunity is inherited independently of two

factors for resistance contained in Marquillo or, if linked, the linkage relation is not very close.

MATERIALS AND METHODS

Parental Varieties

Four varieties of *Triticum vulgare* Vill. were used as parents in the several crosses studied. Marquis is a hard red spring variety of high quality. It is susceptible to stem rust in the field when grown in the spring-wheat region.

Kota is a hard red spring wheat adapted to the less humid sections of the spring-wheat region. It appears somewhat resistant to numerous physiologic forms of rust under field conditions.

Kanred is a hard red winter wheat of high quality when grown in the winter-wheat region. It is immune from several different physiologic forms of stem rust. In the field, however, when certain other forms are present, it may rust quite heavily.

Hybrid No. 1410 is a selection from the cross between Marquis and Kanred. It is a bearded spring wheat which heads normally from three to four weeks later than Marquis.

Crosses

The progeny of four crosses were used in the present inheritance studies. A report was made by the writer (1), in 1923, of the results obtained from a preliminary study of the F_1, F_2, and F_3 hybrids from a cross between Marquis and Kanred. Further studies have been made of growth habit in the F_3 and F_4. A number of selections from this cross, in which were combined the spring growth habit of Marquis with the immunity of Kanred from certain forms of rust, were bulked for rod row trials of yielding ability and other characteristics. These selections were grown as bulks for three years and then again tested in the greenhouse for their reaction to particular physiologic forms as a check on the determinations made in the F_3.

The F_3 plants from a second cross between Marquis and Kanred were studied for their growth habit only. The seed from F_2 plants grown at Chico, California, was received by the writer from J. A. Clark, of the Office of Cereal Crops and Diseases, United States Department of Agriculture. The seed of the F_1 plants was sown in the fall at Chico and, owing to the mild climate, all of the plants survived the winter. There was no elimination of the non-hardy segregates. Under these conditions all of the plants headed and produced seed. This material then represents a true random F_2 population for both the spring and winter types. In the presentation

of the results in the discussions which follow, the Marquis × Kanred F_2 plants and their progeny will be referred to as the "random" material in order to differentiate it from that originating at St. Paul.

A cross between Kota and Kanred was made by V. H. Florell, at Davis, California, in 1919. The F_1 was grown by the hybridizer at the same place in the following year. Some of the F_1 plants were harvested and sent by J. A. Clark to L. R. Waldron at Fargo, North Dakota, where an F_2 was grown from spring sowing in 1921. The writer gratefully acknowledges the receipt of the F_2 material. The inheritance of growth habit in the F_2, F_3, and F_4, and the rust reaction in the F_3 and F_4 were studied in this cross.

A study of the inheritance of rust reaction in a cross between Marquis and Kota wheats was made in 1923 (15). Studies were made in the greenhouse of the reaction of the seedling plants to physiologic forms 19 and 21. In the present study it seemed desirable to learn the reaction of the hybrids from this cross to physiologic form 1.

The fourth cross studied was a backcross of a late-heading F_4 hybrid segregate from Marquis × Kanred to the Marquis parent. In this cross the F_1 to the F_3 inclusive were studied for growth habit and time of heading.

Characters Studied

Two characters, growth habit and rust reaction, received the chief consideration in this study. Growth habit, as used in these studies, is meant to indicate that general characteristic which differentiates true spring varieties from true winter varieties in their ability to produce heads normally when sown in the field in the spring of the year (Fig. 1). The record of growth habit of each individual plant was made by noting the date of emergence of the first head of each plant. In order to facilitate observations, the seeds were sown at intervals of three inches, in rows one foot apart.

The time during which heading took place was divided into weekly periods. One week from the day on which the first plant produced a head tags were placed on all plants on which one or more heads had emerged. These comprised the first class and included the Marquis checks. One week later tags were attached to all plants which had headed since those of class 1. These constituted the second class. This process was continued until no more plants headed. Those plants which did not head were classed as of true winter type. With the exception of the summer of 1925, all of the winter parent controls failed to head, thereby falling into the same class as the winter hybrids.

The rust studies were made on inoculated seedlings in the greenhouse and on plants growing in the field under an artificial epidemic of black

stem rust. The experiments in the greenhouse with the seedling plants were made with urediniospore cultures of known physiologic forms. The cultures were obtained from E. C. Stakman and M. N. Levine. The first leaf was inoculated when about two inches tall. In the field the plants were grown under an artificial epidemic produced by spraying the plants with a suspension of urediniospores of several different physiologic forms of stem rust.

The histological studies were made in an attempt to find some possible morphological character which might be correlated with the differences in rust reaction of Marquis, Kota, and certain Marquis × Kota hybrids to physiologic form 1. The primary structural characteristic under observation was the ratio of sclerenchymatous tissue to collenchymatous tissue in seedling leaves and peduncles of the plants. The peduncles were collected when the heads were in the soft-dough stage. Pieces about two inches long, taken one inch below the head, were killed and cleared in aceto-alcohol. The seedling leaves were collected when the rust pustules were well developed (18 days) and then treated in the same manner as the peduncles. Sections about 10–15 μ thick were cut in pith by means of a sliding razor. Safranin and Delafield's hematoxylin were used to differentiate the lignified and cellulose tissues.

EXPERIMENTAL RESULTS

Growth Habit

Factors Involved. The rate of growth of winter wheat, when fall-sown in the field, is retarded by the lower temperatures concurrently with the advance of the season. Klages (24) and Maximov and Poiarkova (27) have shown that when winter wheat is sown in the greenhouse, in the fall of the year, there is one continuous growth curve, with no indication of a period of dormancy. Koernicke (25) states that both spring and winter wheats undergo a pause in the course of development and that it is short in the case of spring wheats and long in the case of winter wheats.

Marquis, Kota and Kanred wheats showed no great difference in the form of growth curves when sown in the greenhouse in October. Kota and Kanred headed at the same time and Marquis only a few days earlier. These results are in agreement with those of Klages and of Maximov and Poiarkova. They demonstrate that the rhythm in development of winter wheat, when fall-sown in the field, is enforced and not voluntary or inherent. These same writers also demonstrated that the exposure of fall-sown wheat to freezing temperatures is not essential to culm and flower formation. They grew a number of varieties that had been exposed to low temperatures in the early stages of development. These plants were later brought into the greenhouse, and they all headed on practically the same date as the unexposed plants.

Winter and spring wheats, when sown in the greenhouse in the late winter or early spring, do not show this same relationship. When spring-sown, Marquis and Kota will show a continuous growth curve and head normally. Kanred, on the other hand, will remain dormant for several months or succumb to the high summer temperatures before heading. Klages (24) likewise found considerable differences between spring and winter wheats in their ability to produce heads when grown in the greenhouse from seed sown in late winter or early spring.

On several occasions cultures of Marquis and Kanred were transplanted to the field early in the spring, so that they were exposed to temperatures several degrees below freezing. After having been exposed in this manner, Kanred headed normally and was only a few days later in heading than Marquis. Kanred remained dormant, however, when transplanted from the greenhouse to the field later in the spring after freezing temperature periods had passed. Marquis transplanted at this time headed normally, as did the cultures of both Marquis and Kanred transplanted earlier. Call and Salmon (4) and Jensen (22) have observed that winter wheat will head normally, if the seed is sown in the late winter or early spring, in those years when the season opens very early and is followed by low temperatures. From these results and those of the writer, it appears that the environmental conditions in the late winter or early spring are such that late-sown (February or March) winter wheat naturally goes into a resting or dormant period, and that an awakening from this dormancy can be brought about by exposure to low temperatures.

The studies on growth habit, of all of the hybrid material reported in this paper, were made on plants grown from spring-sown seed in the field. Numerous check rows both of the spring and winter parental varieties were grown in the experimental plots.

Marquis × *Kanred.* None of the F_1 plants were grown from spring-sown seed; consequently an accurate determination of their growth habit could not be made. The seed was sown in the fall of the year and, owing to the mildness of the season, only two out of 75 plants were killed during the winter. These plants headed at approximately the same time as Kanred.

All of the seed from the F_1 plants was sown in the spring of the following year. There were 5,253 F_2 plants, of which 442, or 8.4 per cent, were of the winter type. Of the 4,811 plants which headed during the summer, 980 headed in the first period at the same time as the spring parent. In the second weekly period, 1,503 plants headed, which proved to be the modal class for the F_2 population. From here on there was a gradual decrease in the number of plants in each class until the eighth heading period, in which there were only 19. If it were assumed that all of the plants which headed during the summer were spring types, there would be a ratio of approxi-

mately 11 spring types to 1 winter. The distribution of the parental varieties and the F_2 in the various classes for growth habit are shown in table 1.

TABLE 1.—*The growth habit of Marquis, Kanred, and F_2 and F_3 hybrid plants from a cross between Marquis and Kanred*

Material	Weekly heading periods									Total number of plants	Per cent winter types
	1	2	3	4	5	6	7	8	Winter		
Marquis	131	0	0	0	0	0	0	0	0	131	0.0
Kanred	0	0	0	0	0	0	0	0	111	111	100.0
F_2 total	980	1503	883	568	417	313	128	19	442	5253	8.4
F_3 1	888[a]	178	37	14	3	1	1	0	5	1127	0.4
F_3 2	501	282	78	65	42	18	7	10	62	1065	5.8
F_3 3	319	392	214	106	39	33	16	7	41	1167	3.5
F_3 4	163	140	286	178	39	30	12	13	94	955	9.8
F_3 5	63	102	79	118	68	52	5	1	102	590	17.3
F_3 6	13	42	37	38	31	32	10	15	90	308	29.2

[a] The model class in each F_3 group is underlined

The segregation in F_2 was rather complex and indicated that several factors were concerned with the inheritance of spring and winter growth habit. The F_2 data are fitted to several theoretical ratios and reproduced in table 2.

TABLE 2.—*Segregation in F_2 of Marquis × Kanred cross for spring and winter types, and calculation of goodness of fit to 3:1, 13:3, 15:1, and 63:1 ratios*

Theoretical ratio	Type	Spring	Winter	Deviation	Probable error	Deviation P. E.
3:1	Observed	4811.0	442.0	871.3	21.2	41.1
	Calculated	3939.7	1313.3			
13:3	Observed	4811.0	442.0	542.9	19.1	28.4
	Calculated	4268.1	984.9			
15:1	Observed	4811.0	442.0	113.7	11.9	9.6
	Calculated	4924.7	328.3			
63:1	Observed	4811.0	442.0	360.0	6.1	59.0
	Calculated	5171.0	82.0			

Odds = very large in all cases.

The odds against the occurrence of deviations as great or greater than the one obtained are very large for all of the ratios. The calculations for all four ratios are presented in order to show that by comparison it is evi-

dent that the theoretical expected 15:1 ratio gives the best fit to the observed ratios. Two of the 173 F_2 plants, which were classified as spring types, were homozygous for winter habit in F_3. If these plants were truly winter types, which headed due to the favorable conditions for growth late in the season, then a correction should be made accordingly in the F_2 classification for growth habit. One and two tenths per cent, or 58, of the 4,811 plants which headed, should properly be considered as winter types on the basis of their breeding behavior in F_3. The corrected ratio is 4,753 spring types to 500 winter types. This is a wide deviation from the theoretical expected 15:1 ratio. The calculation of goodness of fit for these numbers gives very large odds against the occurrence of a deviation as great as, or greater than the observed one on the basis of random sampling. Such a ratio could be accounted for by assuming that growth habit is governed by two pairs of factors for spring habit which are dominant over their recessive allelomorphs, the factors for winter habit. The breeding behavior of F_2 plants, representing various heading classes in the F_3, should throw some light on the correctness of this hypothesis.

Sixty-five F_2 plants were selected in 1922 and studied for their breeding behavior in F_3. Ten plants were taken from each of the first five heading periods, nine from the sixth, and six from the seventh. In the following year, 1923, 108 additional F_2 plants were selected from the various heading periods for a further study of their breeding behavior in F_3.

The distribution of the F_3 plants in the various classes for heading was somewhat similar to that of the F_2. Of the 5,212 plants grown, 394, or 7.6 per cent, were winter types. There is also a close relation between the heading period of the F_2 plants and the percentage of winter types produced by their progeny. The distribution of the plants in F_3 and the percentage of winter types produced in the progeny of F_2 plants belonging to separate heading periods are given in table 1. In the progeny of the F_2 plants belonging to class 1, 0.4 per cent of the plants were winter types; in class 2, 5.8 per cent; in class 3, 3.5 per cent; in class 4, 9.8 per cent; in class 5, 17.3 per cent; and in class 6, 29.2 per cent.

F_2 phenotype and frequency	F_3 genotype and frequency	Behavior in F_3
15 spring	1 AA BB	Breeds true for spring habit
	2 Aa BB	Breeds true for spring habit
	2 AA Bb	Breeds true for spring habit
	4 Aa Bb	15 spring : 1 winter
	1 AA bb	Breeds true for spring habit
	2 Aa bb	3 spring : 1 winter
	1 aa BB	Breeds true for spring habit
	2 aa Bb	3 spring : 1 winter
1 winter	1 aa bb	Breeds true for winter habit

If the two-factor hypothesis suggested in the F_2 is correct, the breeding behavior of the F_3 would be as follows: "A" and "B" are dominant duplicate factors for spring habit; and "a" and "b", their allelomorphs, the recessive factors for winter habit.

The proportions of each type breeding true and segregating in F_3 would be as follows: 7 breeding true for spring habit, 8 segregating for spring and winter types, 1 breeding true for winter habit.

Owing to the failure of the winter types to head, the last group would not normally be expected to appear in the F_3 from the St. Paul material, but would be expected in the random material from Chico.

The F_3 lines were classified according to the three groups mentioned above. As only a certain number of F_2 plants were selected from each of the various heading periods, it was necessary to compute their frequency in these classes on the basis of their actual distribution in F_2. Such calculations will make it possible to compare the proportions of the true-breeding and segregating F_3 lines with the theoretical proportions.

In the second generation there were 980 individuals in the first heading period, which represents 20.5 per cent of the 4,789 F_2 plants that produced seed. In the F_3, 173 lines were grown. Twenty and five-tenths per cent, or 35.5 plants, would be the calculated number for class 1 on the basis of the F_2 frequency distribution. The actual number of F_3 lines grown from seed of class 1 F_2 plants was 29. Of these, 28 were pure for spring habit and one was segregating for growth habit. The corresponding proportions for the calculated number of 35.5 would be 34.3 pure for spring habit and 1.2 segregating for growth habit. Computations were made in a like manner for each heading class. The observed and calculated results are drawn up in table 3. The actual number of plants selected in F_2 and grown in F_3 is given in the first column of each group and the number calculated on the basis of the F_2 distribution is given in the second column.

Only one of the F_2 plants selected from class 1 produced progeny in F_3 which segregated into spring and winter types. In this one line there were 78 springs to 5 winters. Beginning with the plants in the second heading period, there was a gradual decrease in the number of lines which were homozygous for the spring character and a corresponding increase in the number of lines heterozygous for spring and winter types as the seventh heading period was approached. No families were homozygous for the spring habit of growth in either the sixth or seventh heading periods. F_3 lines from plants with such late heading periods were either heterozygous for growth habit or homozygous for winter habit. In general, the later the heading period of the F_2 plants, the larger the percentage of winter types to spring types in the F_3.

TABLE 3.—*The breeding behavior in F_3 of plants belonging to separate F_2 heading groups in crosses between Marquis and Kanred*

F_2 weekly heading period	Number of F_3 lines							
	Grown		Pure for spring habit		Segregating for growth habit		Pure for winter habit	
	Actual	Calculated	Actual	Calculated	Actual	Calculated	Actual	Calculated
1	29	35.5	28	34.3	1	1.2	0	0.0
2	29	54.3	11	20.6	18	33.7	0	0.0
3	30	31.8	18	19.1	12	12.7	0	0.0
4	28	20.6	10	7.4	18	13.2	0	0.0
5	30	15.1	5	2.5	25	12.6	0	0.0
6	21	11.2	0	0.0	21	11.2	0	0.0
7	6	4.7	0	0.0	4	3.1	2	1.6
Total	173	173.2	72	83.9	99	87.7	2	1.6

When calculated on the basis of the F_2 frequency, the 173 F_3 lines gave a fairly close fit to the theoretical expected. There were 83.8 homozygous for spring habit, 87.6 segregating for spring and winter habit, and 1.6 homozygous for winter habit. The results obtained are compared with the theoretical expected by the goodness of fit method and reproduced in table 4.

TABLE 4.—*Summarized data from table 3 fitted to a theoretical 7:8:0 ratio*

Group	Expected ratio	Ratio		O–C	$(O–C)^2$	$\frac{(O–C)^2}{C}$
		Observed	Calculated			
Pure spring	7	83.8	80.7	3.1	9.61	0.119
Segregating	8	87.6	92.3	4.7	22.09	0.239
Pure winter	0	1.6	0.0	1.6	2.56	2.560
	P = 0.2350				$X^2 = 2.918$	

Owing to the presence of the homozygous winter type, the fit is not so close as might otherwise be expected. The two F_3 lines, which were pure for winter habit, were the progeny of very late heading F_2 plants. These may possibly have been winter forms which, under the conditions peculiar to 1922, headed and produced seed. Consequently they were wrongly classified as to growth habit in the F_2. If this possibility is taken into consideration and a correction made accordingly, the recalculated ratios would give a fit to the theoretical expected with a P value of 0.8591. As bad a result, or worse, is expected on the basis of random sampling in about 86.0 per cent of such trials.

The F_2 plants received from Chico were not tagged individually for heading period; consequently a study of the relation between the heading period of F_2 plants and the breeding behavior of their progeny could not be made in this material. The seed from F_1 plants was fall-sown and, owing to the mild climate, all of the F_2 plants survived and produced seed. The F_3 was grown at St. Paul in 1922, in the same field as the first lot of F_2 Marquis × Kanred material.

A larger percentage of the F_3 lines from the random F_2 were homozygous for the winter habit than of the F_3 lines from St. Paul. Seven out of 233, or 3 per cent, of the former were pure winter types; while 1.6 out of 173, or 0.9 per cent, of the latter were pure winter types. Such a difference, or even a greater one, was to be expected in view of the fact that the winter types in F_2 had not been eliminated in the random material as they were at St. Paul when they failed to head and produce seed. There is a difference of only 2.1 per cent, which probably indicates that not all of the winter types were eliminated in the F_2 at St. Paul. The pure winter types were probably the chief ones to be eliminated; consequently it may be assumed that the F_3 represented a fair random sample of the spring types at least.

The breeding behavior of 233 F_2 plants, representing a true random sample, was studied in F_3. There were 120 of these F_3 lines homozygous for spring habit, 106 segregating for spring and winter types, and 7 homozygous for winter habit. The data obtained from this material are compared with the theoretical expected 7 : 8 : 1 ratio by the goodness of fit method and reproduced in table 5.

TABLE 5.—*Segregation in F_3 of Marquis × Kanred cross from a random F_2 for spring and winter habit and calculation of goodness of fit to a 7 :8 :1 ratio*

Group	Expected ratio	Ratio		O–C	$(O–C)^2$	$\frac{(O–C)^2}{C}$
		Observed	Calculated			
Pure spring	7	120	102.2	17.8	316.84	3.100
Segregating	8	106	116.8	10.8	116.64	0.999
Pure winter	1	7	14.5	7.5	56.25	3.879
P = 0.0186					$X^2 = 7.978$	

A study of the data seems to indicate that the primary reason for the poor fit is the lack of winter types in the third group. If the conditions peculiar to that year were especially favorable for heading, some of the winter types may possibly have headed. The result would be that the third group containing the pure winters would be too small and the first group containing pure springs would be too large. The deviations of the

observed from the expected seem to indicate that such was the case; consequently a P value as small as 0.0186 was anticipated in the calculations.

In order to obtain an indication of the nature of the segregation in the heterozygous F_2 plants, each segregating F_3 line was classified as a 15:1 or a 3:1 ratio on the basis of its goodness of fit. The tables of probable errors of Mendelian ratios prepared by the Department of Plant Breeding of Cornell University were used for this purpose. The number of individuals in each class for heading was computed on the basis of the frequency distribution in F_2. The data obtained from these observations and calculations are given in table 6. The observed numbers are given in the first column and the calculated numbers in the second column for each of the ratios.

TABLE 6.—*The breeding behavior in F_3 of heterozygous plants belonging to separate F_2 heading groups in crosses between Marquis and Kanred*

F_2 weekly heading period	Total number		Number of F_3 segregating for a				Percentage segregating 15:1
			15:1 ratio		3:1 ratio		
	Actual	Calculated	Actual	Calculated	Actual	Calculated	
1	1	1.2	1	1.2	0	0.0	100.0
2	18	33.7	17	31.8	1	1.9	94.4
3	12	12.7	10	10.6	2	2.1	83.3
4	18	13.2	7	5.1	11	8.1	38.9
5	25	12.6	7	3.5	18	9.1	28.0
6	21	11.2	3	1.6	18	9.6	14.3
7	4	3.1	0	0.0	4	3.1	0.0
Total	99	87.7	45	53.8	54	33.9	

The calculated number of F_3 lines from the St. Paul material segregating for growth habit is 87.7. There are also 106 such lines in the random material, making a total of 193.7 F_3 lines segregating for spring and winter habit. Of these combined numbers, 98.8 gave 15:1 ratios and 94.9 gave 3:1 ratios. According to the hypothesis suggested, one-half of the heterozygous F_3 lines should be segregating for two factor pairs and give 15:1 ratios, and the other half for a single factor pair and give 3:1 ratios. The results are very close to the expected and are fitted to a theoretical 1:1 ratio in table 7.

The observed results produced a very close approximation to the calculated as is indicated by the fit to the 1:1 ratio.

Another method of analysis was used which helped to bring out the nature of the results. This was a comparison of the observed ratios of individual plants with the calculated in the heterozygous F_3 lines. There

TABLE 7.—*Segregation in heterozygous F_3 lines of Marquis × Kanred cross for 15:1 and 3:1 ratios of spring to winter types and calculation of goodness of fit to a 1:1 ratio*

Ratio class	Number		Dev.	P.E.	D/P.E.	Odds
	Observed	Calculated				
15:1	98.8	96.8	2	4.7	0.4	less than 1:1
3:1	94.9	96.8				

were 970 plants in the F_3 lines from the St. Paul material segregating into 3:1 ratios for spring and winter habit, and 1,988 in those segregating into 15:1 ratios. In the random sample there were 1,698 in the 3:1 ratios and 1,384 in the 15:1 ratios. The results are summarized in table 8, and fitted to their respective theoretical calculated ratios.

TABLE 8.—*Ratios of individual plants in heterozygous Marquis × Kanred F_3 lines segregating for spring and winter habit and their fit to the theoretical calculated*

Expected ratio	Classes	Number		Dev.	P.E.	D/P.E.	Odds
		Observed	Calculated				
St. Paul F_3 material							
3:1	Spring	732	727.5	4.5	9.1	0.5	Very small
	Winter	238	242.5				
15:1	Spring	1847	1863.8	16.8	7.3	2.3	7.3 to 1
	Winter	141	124.2				
Random F_3 material							
3:1	Spring	1258	1273.5	15.5	12.0	1.3	1.6 to 1
	Winter	440	424.5				
15:1	Spring	1290	1297.5	7.5	6.1	1.2	1.4 to 1
	Winter	94	86.5				

In all cases the odds against the occurrence of deviations as great or greater than the observed ones are very small and indicate a good fit between the observed and theoretical calculated ratios.

Early Versus Late Heading. There is a rather close relationship between the heading period of individual plants in the F_2 and that of their progeny in F_3. The progeny of F_2 plants from class 1 had a mode for heading in class 1. The progeny from class 2 plants, likewise, had a mode in class 1 but to a less marked degree. Those from class 3 F_2 plants had a

mode in class 2; class 4 in class 3; and class 5 in class 4. The F_2 plants from class 6 produced progeny which were rather evenly distributed over all classes of heading in F_3 with a mode in the winter group. The heading periods of the F_2 plants and their progeny are given in table 1.

A complete analysis of early and late heading could not be made for all classes, owing to the failure of the winter types to head and also because of the possible influence of the factors for growth habit upon early or late heading.

From a study of the data presented in table 6, it will be noted that there is a fairly close relationship between the heading period of the F_2 plants and their breeding behavior for growth habit in F_3. Those plants in the earlier classes, especially in classes 1 to 3, segregated in the majority of the cases into 15:1 ratios, while the plants in the later classes segregated into 3:1 ratios. It appears that when both of the factors for spring habit are present the heading period is, in general, earlier than when only one of the factors for spring habit is present. This relationship is illustrated by the percentage of F_3 lines segregating for a 15:1 ratio and those segregating for a 3:1 ratio. In class 1 there was only one heterozygous line and it segregated into a 15:1 ratio; in class 2, 94.4 per cent segregated into 15:1 ratios; in class 3, 83.3 per cent; in class 4, 38.9 per cent; in class 5, 28.0 per cent; in class 6, 14.3 per cent; and in class 7, 0.0 per cent. In the last named class there were four heterozygous lines and all of them segregated into 3:1 ratios. These results suggest that there are factors for early and late heading in addition to the factors for growth habit. This is illustrated by the cases where rather early heading is combined with a single factor difference for growth habit. The F_2 plant headed early, and its progeny produced a monohybrid ratio for spring and winter habit. On the other hand, some of the F_2 plants which headed as late as the sixth heading period produced progeny which segregated as dihybrids for spring and winter habit. (See table 6.)

A study of early and late heading in the F_2 and F_3 data failed to reveal any especially significant ratios which might be satisfactorily explained on the basis of a single factor for early and late heading in addition to the factors for spring and winter growth habit. However, if it were assumed that there are two factor pairs for earliness and that they are independent of the growth habit factors, a fairly satisfactory fit to a theoretical 189:67 ratio can be obtained. When all four factors are present, the plant might be expected to head as early as the first or second period, and with any one of the four absent and its allelomorph for lateness or winter habit present, the plant might be expected to head as late as the third or fourth period. Considering, then, the F_2 plants in classes 1 to 4 as the earlier group and

those in the remaining classes as the later group, a fairly close approach to the theoretical expected is obtained. An outline of the factorial hypothesis would be as follows in F_2:

Numerical proportions	Genotype	Phenotype	
81	A B C D	Early spring	189 early
27	A B C d		
27	A B c D		
27	A b C D		
27	a B C D		
9	A B c d	Late spring	67 late
9	A b C d		
9	A b c D		
9	a B C d		
9	a B c D		
9	a b C D	Early winter	
3	A b c d	Late spring	
3	a B c d		
3	a b C d	Late winter	
3	a b c D		
1	a b c d		

The F_2 phenotypic ratio of early and late heading plants is reproduced in table 9 and calculated for a goodness of fit to a theoretical 189 : 67 ratio.

TABLE 9.—*Segregation in F_2 for early and late heading in crosses between Marquis and Kanred, and calculation of goodness of fit to a 189:67 ratio*

Heading class	Ratio		Dev.	P.E.	D/P.E.	Odds
	Observed	Calculated				
Early 1–4	3934	3878	56	21.2	2.6	11.6 : 1
Late 5–9	1319	1375				

The odds against the occurrence of a deviation as great, or greater than the one obtained on the basis of random sampling are 11.6 : 1. The four-factor hypothesis, therefore, explains the results in a satisfactory manner. Further study is necessary to substantiate this hypothesis.

A number of F_3 plants were selected from both homozygous and heterozygous lines in the first six heading periods for a study of breeding behavior in F_4. The results are given in table 10 where the plants are classified according to their heading period in F_3, the breeding behavior of the F_3 line from which they were selected, and the segregation in F_4.

Of the 37 plants selected from F_3 lines homozygous for the spring character, 36 produced F_4 progeny which were likewise homozygous for the

TABLE 10.—*The growth habit of F_4 lines from plants belonging to separate F_3 heading groups from a cross between Marquis and Kanred*

F_3 weekly heading period	Growth habit of F_3 lines	Number of F_4 families			
		Grown	Homozygous for spring habit	Heterozygous for growth habit	Homozygous for winter habit
1	Homozygous spring	8	8	0	0
2	Homozygous spring	9	9	0	0
2	Heterozygous	10	6	4	0
3	Homozygous spring	5	5	0	0
3	Heterozygous	10	7	3	0
4	Homozygous spring	10	10	0	0
4	Heterozygous	10	7	3	0
5	Homozygous spring	6	5	1	0
5	Heterozygous	7	5	2	0
6	Heterozygous	6	5	1	0
Total		81	67	14	0

spring character. One of the F_3 plants selected from the fifth heading period produced progeny which segregated into 15 spring types to one winter type.

There were 43 plants selected from F_3 lines heterozygous for growth habit, and 30 of these produced progeny which were homozygous for growth habit in F_4. Thirteen were heterozygous for growth habit, ten of which produced 15 : 1 ratios and three of which produced 3 : 1 ratios for spring and winter types.

The proportion of spring types to winter types in F_4 was much greater than in F_3. This is undoubtedly the result of selection in F_3. In the heterozygous lines, plants with the larger number of factors for spring habit would be most likely to mature seed and consequently the winter types would be expected to decrease proportionally by selection.

Kota × Kanred. The F_2 plants from the Kota × Kanred cross, grown at Fargo, North Dakota, were not studied individually for period of heading. The plants were pulled at harvest time and then sorted into spring and winter types. There were 1,305 plants, 1,177 of which headed and 128 of which did not head. This proportion of spring to winter types is very close to that obtained in the F_2 from the Marquis × Kanred cross grown at St. Paul, Minnesota. Of the former, 9.8 per cent of the F_2 plants were winter types; and of the latter, 8.4 per cent of the F_2 plants were winter types. The data from the Kota × Kanred cross are reproduced in table 11 and fitted to a theoretical 15 : 1 ratio.

TABLE 11.—*Segregation in F_2 of Kota × Kanred cross for spring and winter types, and calculation of goodness of fit to a theoretical 15:1 ratio*

Class	Ratio		Dev.	P.E.	D/P.E.	Odds
	Observed	Calculated				
Spring	1177	1223.4	46.4	5.9	7.86	Very large
Winter	128	81.6				

On the basis of random sampling the odds against the occurrence of a deviation as great or greater than the one observed are very large. This is a slightly better fit than that obtained from the Marquis × Kanred cross, however, where the deviation divided by the probable error was 9.6. Calculations of goodness of fit to theoretical 3:1, 13:3 and 63:1 ratios were also made as in the previous cross, but here again the 15:1 ratio gave the best fit between the observed and theoretical expected ratios.

The plants were not classified as to weekly heading periods in the F_2, as in the former cross. Thirty-nine plants, all of the progeny that produced seed from a single F_1 plant, were grown in the F_3. Nineteen of the F_2 plants proved to be pure for spring habit in F_3, and twenty segregated for both spring and winter types. No pure winter types were found. The same factor hypothesis is being used to explain the breeding behavior of the hybrid progeny in this cross as in the cross between Marquis and Kanred. The data are reproduced in table 12 and fitted to a theoretical 7:8:0 ratio.

TABLE 12.—*The breeding behavior of F_3 plants representing a random F_2 population from a cross between Kota and Kanred, and calculation of goodness of fit to a theoretical 7:8:0 ratio*

Group	Expected ratio	Ratio		O–C	$(O–C)^2$	$\frac{(O–C)^2}{C}$
		Observed	Calculated			
Pure spring	7	19	18.2	0.8	0.64	0.035
Segregating	8	20	20.8	0.2	0.04	0.002
Pure winter	0	0	0.0	0.0	0.00	0.000
P = very large					$X^2 = 0.037$	

On the basis of random sampling such a variation would be expected in about 90 per cent of such trials. These results indicate that the two-factor hypothesis giving a 15:1 ratio in the F_2 is probably the correct one.

The 20 plants segregating in F_3 for both spring and winter types were analyzed for the proportions of individuals producing 15:1 ratios to those producing 3:1 ratios. There were 9 of the former and 11 of the latter.

This is very close to the expected 1:1 ratio and indicates that the observed results are in close agreement with the theoretical expected.

A comparison in this cross was made also of the observed ratios of individual plants with the expected ratios in the heterozygous F_3 lines. There were 517 plants in the F_3 lines segregating for 3:1 ratios for spring and winter habit and 689 in those segregating for 15:1 ratios. The results are summarized in table 13 and fitted to their respective theoretical expected ratios.

TABLE 13.—*Ratios of individual plants in heterozygous Kota × Kanred F_3 lines segregating for spring and winter habit and their fit to the theoretical calculated*

Expected ratio	Classes	Number		Dev.	P.E.	D/P.E.	Odds
		Observed	Calculated				
3:1	Spring	395	387.8	7.2	6.6	1.1	1.2 to 1
	Winter	122	129.2				
15:1	Spring	639	645.9	6.9	4.3	1.6	2.6 to 1
	Winter	50	43.1				

On the basis of random sampling the odds against the occurrence of deviations as great or greater than the observed ones are very small for both ratios and indicate a good fit between the observed and theoretical expected ratios.

The results obtained from a study of the cross between Kota and Kanred are in close agreement with the results obtained in the study of inheritance of growth habit in the cross between Marquis and Kanred. The two-factor hypothesis fairly satisfactorily explains the breeding behavior. Marquis and Kota may both be designated as having the two dominant factors for spring habit, "A" and "B," and Kanred their recessive allelomorphs for winter habit, "a" and "b."

Marquis × (Marquis × Kanred). An attempt was made to throw some light on the genotypic constitution of one of the late-heading hybrid selections by backcrossing it to Marquis, the spring parent. Such a late-heading spring type would probably contain at least one of the factors for spring habit (A or B) and the recessive factors for late heading (a and b).

The F_4 Marquis × Kanred spring hybrid number "1410" which has a heading period four weeks later than Marquis was used as the hybrid parent in this cross. It was pure for spring habit in F_3 and has not thrown any winter types in the four years it has been grown (Fig. 1). The heading date of each individual plant was noted and classified as in the Marquis × Kanred cross. The data thus obtained are presented in table 14.

There were 104 F_1 plants grown in the field. They all headed in the same weekly period as the Marquis parent, indicating a complete dominance of the early over the late heading. The genotype of Marquis may be designated as AA BB CC DD, and that of the hybrid selection may be designated

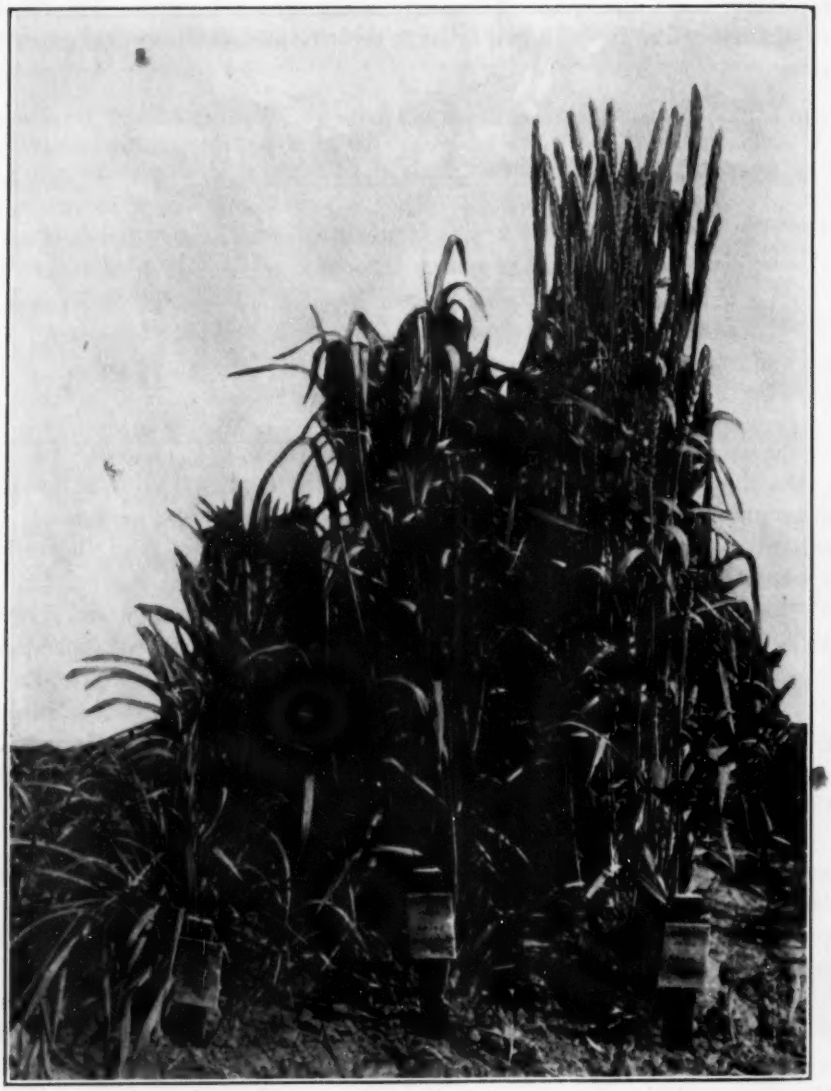


FIG. 1. The growth habit of Kanred, Marquis × Kanred hybrid 1410, and Marquis at the time Marquis was fully headed.

TABLE 14.—*The growth habit of Marquis, Kanred, Hybrid 1410 and F_2 and F_3 hybrids from a backcross between Marquis and Hybrid 1410*

Year	Material	Weekly heading periods							Total number of plants
		1	2	3	4	5	6	Winters	
1924	Marquis	55	3	0	0	0	0	0	58
	Kanred	0	0	0	0	0	0	13	13
	Hybrid 1410	0	0	5	17	7	4	0	33
	F_1 total	104	0	0	0	0	0	0	104
	F_2 total	43	94	24	5	1	0	12†	179
1925	Marquis	359	48	0	0	0	0	0	407
	Kanred	0	0	0	0	1	6	171	178
	Hybrid 1410	0	14	139	135	21	0	0	309
	F_2 total	3184	2774	682	78	2	0	0	6720
	F_3 1	772	306	27	2	0	0	0	1107
	F_3 2	353	807	311	48	11	0	0	1530
	F_3 3	2	27	24	6	0	0	0	59
	F_3 4	0	4	31	10	0	0	0	45
	F_3 5	0	5	25	9	0	0	0	39

as AA bb cc dd. The F_1 will then be AA Bb Cc Dd, and would be a spring type with an early heading period.

A number of F_1 plants were grown to maturity in the greenhouse during the winter of 1923–24. The seed from these plants was late in maturing and when sown in the field produced plants which grew poorly. They appeared to be rather unsatisfactory material upon which to make a study of growth habit and heading.

The following summer, an F_2 population of 6,720 plants was grown from seed produced by the F_1 plants raised in the field. Of this large number, not one remained dormant as a true winter wheat. The season was unusual, as was indicated by the heading of several of the Kanred check plants. This was the first instance in the five years during which these studies were carried on that any of the Kanred plants headed when grown from spring-sown seed. Under such conditions it is rather difficult to conclude whether any true winter types were produced by the backcross. There is an overlapping in the frequency distribution of the hybrid plants and Kanred in class 5. One Kanred plant headed at that time, and six headed in the sixth period. Seed from the Kanred plants that headed was harvested and sown in the fall. The plants survived the winter and appeared to be typical of the variety.

Owing to the heading of some of the winter parent plants, it seemed that probably the season was unusually favorable for the development of this character. The variability of the parental varieties in this particular

year is so great that one could scarcely expect to find the hybrids fitting into any particular classification. Marquis plants headed over a period of two weeks, the late spring hybrid over a period of four weeks, and Kanred over a period of at least three weeks. This great variability may possibly be accounted for by the flooding of that portion of the field in which this material was grown, on two separate occasions, the first one at the time the plants were beginning to send out shoots and the second during the first weekly heading period. The F_3 plants, which were the progeny of the F_2 plants grown in 1924, were also subject to these same conditions and consequently not amenable to a detailed analysis from which very definite conclusions can be drawn. The summer of 1925 was an illustration of how erratic the environmental conditions can be in their influence upon such characters as time of heading and growth habit. In general, the results do seem to show that a large proportion of the segregates in the F_2 were of the earlier group and that probably no true winter types were produced in the F_2 or F_3 progeny from this cross.

Seedling Posture. There are distinct differences in the posture of the leaves of various wheat varieties in the early stage of development of the plant. Hardy varieties of winter wheat are commonly believed to have a recumbent habit in contrast to an upright one characteristic of most spring varieties (31). This is generally true, but there are some outstanding exceptions, such as Padui, which has a marked upright leaf posture and is also winterhardy. Dawson's Golden Chaff and Red Cross are reported by Klages (24) as being erect types and yet quite hardy.

The 233 F_3 families representing a true random sample from a Marquis × Kanred cross were studied for this character in the field. The plants were grown from spring sowings. The notes on posture were made early in June and recorded in five classes or types. The leaves of Kanred, the winter parent, were prostrate and the plants were designated as "type 1." The leaves of Marquis, the spring parent, were erect and the plants were designated as "type 5." Intermediate degrees of erectness were designated as types "2," "3," and "4." Many of the F_3 lines were still segregating for this character, in which case estimated averages were made to represent the line as a whole. Consequently, it is not known whether the F_3 lines were homozygous or heterozygous for this character.

Later in the season each F_3 line was classified as being either homozygous for spring habit, heterozygous for spring and winter habit, or homozygous for winter habit. The date of heading was not taken on each individual plant. The relationship between the two characters is shown by the amount of association between the plants in the various classes of seedling posture and their distribution in the three groups for growth habit. The data are presented in table 15.

TABLE 15.—*Relation between seedling posture and growth habit in 233 F_3 lines from a cross between Marquis and Kanred*

Material	Growth habit	Seedling posture[a]				
		1	2	3	4	5
Kanred	Winter	4	0	0	0	0
Marquis	Spring	0	0	0	0	16
117 F_3 lines	Spring	0	3	56	39	19
109 F_3 lines	Spring and winter	0	18	82	6	3
7 F_3 lines	Winter	0	4	3	0	0

[a] 1 = prostrate; 2 = semi-prostrate; 3 = intermediate; 4 = semi-erect; 5 = erect.

None of the F_3 lines was homozygous for the prostrate condition typified by the Kanred parent plants, while a number of lines had an upright posture similar to that of the Marquis parent. There were several individual plants in some of the lines heterozygous for this character, however, which had a prostrate position similar to that of the winter parent. A few hybrids with a spring growth habit approached closely the posture of Kanred. The hybrids with a winter growth habit were either semi-erect or approached the winter parent in posture. There appears to be only a general relationship between growth habit and seedling posture in the hybrids. Consequently the utilization of this characteristic as a criterion in the determination of spring and winter wheats, even in this cross, could only be in a relative way.

RUST REACTION

Marquis × Kanred. The greenhouse study of the inheritance of rust reaction in crosses between Marquis and Kanred was made on the F_3 and F_7 seedlings. In this cross the infection types of the parental material and the hybrids were so distinctly different that to obtain the numerical ratios, after infection, was simply a matter of mechanical assortment and counting of the number of individuals in each class. Macroscopically, at least, there were no essential variations in reaction. The parental material and all of the hybrids were either immune from (type 0) or completely susceptible to (type 4) *Puccinia graminis tritici*, form 1 (Fig. 2). As the parents are at the extremes in the range of infection types for this particular form of rust, no transgressive segregation was observed. The Marquis parent and susceptible hybrid plants produced large, vigorous, and confluent uredinia.

The plants of ten F_3 lines from each of the first six classes and five families from the seventh class for growth habit were tested for their reaction to physiologic form 1. Of the 65 lines tested, 23 were homozygous for

immunity, 32 were heterozygous for immunity and susceptibility, and 10 were homozygous for susceptibility. The F_2 data are reproduced in table 16, and fitted to a theoretical 1:2:1 ratio.

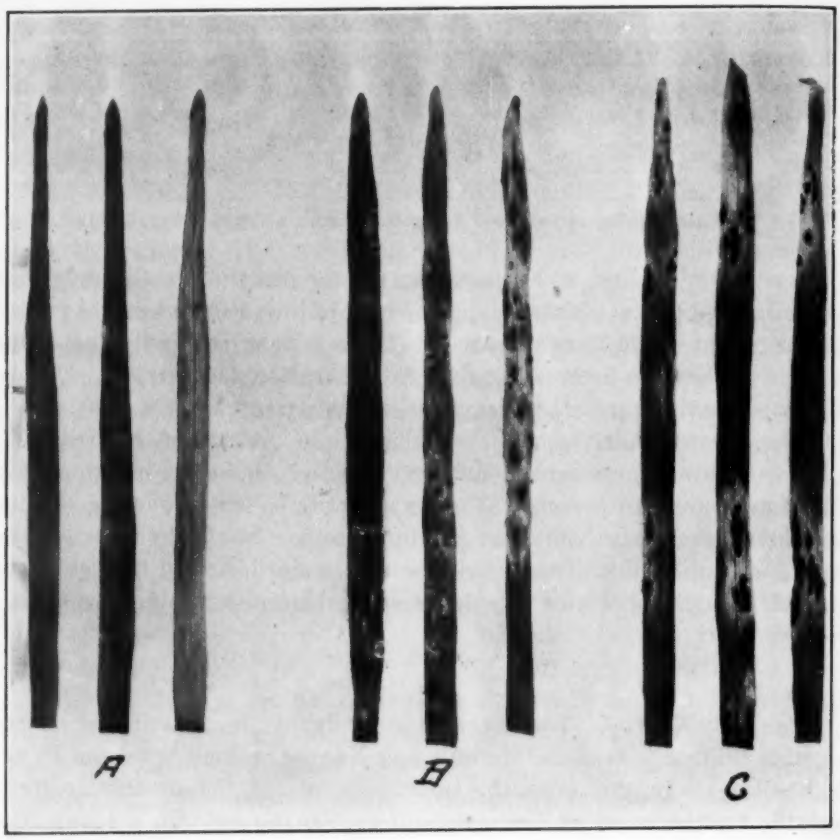


FIG. 2. A multiple allelomorphic series for rust reaction of Kanred (A), Kota (B), and Marquis (C) to *Puccinia graminis tritici* form 1.

As bad a result, or worse, is expected on the basis of random sampling in about 7 per cent of such trials. This fit is not especially close but suggests that the results may be accounted for by a single pair of allelomorphic factors. When the immune and heterozygous groups are combined, there is a close approach to the expected 3:1 ratio. The deviation from the theoretical calculated is 6.2 ± 2.35. The odds against the occurrence of such a deviation being due to random sampling are 11.58:1. This fit is quite satisfactory and suggests that the results may be accounted for by a single pair of allelomorphic factors, dominant for immunity in Kanred and

TABLE 16.—*Segregation of rust reaction in F_3 of a cross between Marquis and Kanred and calculation of goodness of fit to a 1:2:1 ratio*

Class	F_3 lines		O–C	$(O–C)^2$	$\frac{(O–C)^2}{C}$
	Observed	Calculated			
Immune	23	16.2	6.8	46.24	2.854
Heterozygous	32	32.5	0.5	0.25	0.008
Susceptible	10	16.2	6.2	38.44	2.373
Total	65	64.9			
	P = 0.0745				$X^2 = 5.235$

recessive for susceptibility in Marquis. Additional data on this factorial relationship were made available from a study of the ratios of immune to susceptible plants in the heterozygous F_3 lines.

There were 1,020 plants in the heterozygous F_3 lines, 798 of which were immune from rust and 222 of which were susceptible. This is a deviation from the expected monohybrid ratio of 33.0 ± 9.33. A number of the plants escaped infection, as was indicated by the susceptible parent used as a check and by reinoculating some of the plants which had escaped infection. The number which had escaped infection was subtracted from the immune group and added to the susceptible group. A corrected ratio of 767 immune to 253 susceptible was obtained. This has a deviation from the theoretical calculated of 2.0 ± 9.33, which is a very close fit to the expected monohybrid ratio. These results add further proof to the hypothesis that a single pair of genetic factors governs the reaction to physiologic form 1, and that the factor for immunity is dominant to the allelomorphic factor for susceptibility.

The F_3 lines from the different heading periods showed similar numerical relations between the immune and susceptible plants, regardless of their respective times of heading. Rust reaction was independent of growth habit and heading period. Many F_3 and F_4 lines were obtained which are homozygous for the spring habit of growth and immune from infection by form 1. Several of these lines were then studied for their reaction to other physiologic forms of stem rust from which Kanred is immune. The hybrids which were immune from form 1 were likewise found to be immune from the other forms, and it was definitely demonstrated that the immunity of Kanred from several physiologic forms of stem rust was governed by a single pair of genetic factors.

Several hundred of the F_3 lines which appeared to be homozygous for the immune reaction of the Kanred parent were subsequently grown in the field. Fifty-three of these lines which appeared to be desirable spring

wheats were later given an extensive test in the rod row nursery. In the seventh generation they were again grown in the greenhouse and a determination made of their reaction to several of the same physiologic forms of stem rust used previously. Kanred is immune from five of these forms, namely, 1, 9, 17, 19, and 21, and susceptible to forms 3 and 18. Marquis is susceptible to all of these forms, except 19, to which it is resistant. Each hybrid line was given three trials by inoculating three separate sets of plants with each form. There were 12 to 15 seedlings in each set, making an average of 40 seedlings per line.

Fifty of these lines were immune from forms 1, 9, 17, 19, and 23. Two were heterozygous for immunity and susceptibility, and one was susceptible. Approximately 10,000 seedlings were inoculated with the five forms from which Kanred is immune. Of this number, 11, or 0.11 per cent, were susceptible. These exceptions are probably due to natural crosses with some susceptible variety. If such is the case, the cross must have occurred not later than the fifth generation, as immunity is dominant to susceptibility. Marquis checks were grown in every fifth plat in the rod row experiments in the field. Even though the susceptible parental checks were outnumbered five to one and a correction was made strictly on a numerical basis, there would be one-half of one per cent natural crossing between varieties. The rod rows are carefully rogued each year, thus insuring the elimination of a large number of the natural crosses; consequently this figure is much below the one usually given for wheat (17). There is also the possibility that the presence of some of the aberrant seedlings was due to mechanical mixtures.

There was a ratio of 245 immune plants to 108 susceptible in the two F_7 heterozygous hybrid lines. If the plants were heterozygous at the time the individual plants were bulked for the rod rows, it would be difficult to state what proportions of immune and susceptible plants were combined. If they were immune and a natural cross took place in one of the earlier generations, one would not expect such a large percentage of susceptible plants.

The results further substantiate the belief that the reaction to one of several forms of rust from which Kanred is immune may be taken as a criterion of immunity from the remainder of the forms governed by the same factor.

Kota × *Kanred*. The inheritance studies of reaction to form 1 in crosses between Kota and Kanred were made from the F_3 seedlings. Kanred is immune from this rust form and Kota is moderately resistant (Fig. 2). While the infection types of these parental varieties were quite distinct and uniform, some of the F_3 hybrids which were supposedly like Kota did fluctuate somewhat and produce a "type 4" infection. The Kota parent

in this particular experiment did not show any such variation in susceptibility, but it has been so reported by other investigators (34). The increased susceptibility of certain hybrids might be accounted for by a factorial recombination which resulted in transgressive segregation or as differences due to environmental fluctuation.

The inoculations were repeated on a second series of plants, and in this case practically all of the hybrids, which had previously given a "type 4" infection, produced a "type 3" infection similar to that of Kota. In only one of the 104 F_3 lines did the plants appear to have a consistently higher degree of susceptibility. Such an F_3 line may possibly be the result of some recombination of minor modifying factors. The evidence available to substantiate such an hypothesis is meager and would be difficult to obtain. One aberrant line out of 104 could readily be accounted for by a natural cross with some susceptible variety in the F_1. These results seem to indicate that the reaction of some of the Kota × Kanred F_3 lines was subject to variations in rust reaction due to certain uncontrolled environmental conditions. In summarizing the results from this cross, the few variations of the susceptible hybrids from the typical "type 3" infection were considered as environmental fluctuations.

From each of 104 F_3 lines, 30 seedlings were inoculated with physiologic form 1. Of these F_3 lines, 30 were homozygous for the immune reaction, 48 were heterozygous and 26 were homozygous for the moderately resistant reaction. The F_2 data are presented in table 17 and fitted to a theoretical 1:2:1 ratio.

TABLE 17.—*Segregation of rust reaction in F_3 of a cross between Kota and Kanred and calculation of goodness of fit to a 1:2:1 ratio*

Class	F_2 lines		O–C	$(O–C)^2$	$\frac{(O–C)^2}{C}$
	Observed	Calculated			
Immune	30	26	4	16	0.615
Heterozygous	48	52	4	16	0.308
Moderately resistant	26	26	0	0	0.000
Total	104	104			
P = 0.5598					$X^2 = 0.923$

As bad a result, or worse, is expected on the basis of random sampling in about 56 per cent of such trials. Additional data on this factorial relationship were made available from a study of the ratios of immune to moderately resistant plants in the heterozygous F_3 lines.

There were 1,246 individual plants in the 48 heterozygous F_3 lines. Of this number, 939 were immune and 307 were moderately resistant. The

number expected on the basis of a 3 : 1 ratio would be 934.5 immune to 311.5 moderately susceptible. This is a deviation of the observed from the theoretical calculated of 4.5 ± 10.24. The odds against the occurrence of such a deviation due to random sampling are less than 1 : 1. These results add further proof to the hypothesis that a single pair of genetic factors governs the reaction to physiologic form 1 in the cross between Kota and Marquis. The factor for immunity in Kanred is dominant to the allelomorphic factor for moderate resistance in Kota.

The inheritance of reaction to physiologic form 1 in the Kota × Kanred cross appears to be rather closely related genetically to the inheritance of reaction to this same form of rust in the Marquis × Kanred cross. In the latter the immunity of Kanred dominated the complete susceptibility of Marquis. In the Kota × Kanred cross the immunity of Kanred likewise dominated the moderate resistance of Kota. The question naturally arises, are these factors for susceptibility in Marquis and moderate resistance in Kota allelomorphic? A study of the reaction of Kota × Marquis hybrids to the particular form of rust was made in an attempt to obtain further information on the factorial relationship of these two varieties.

Marquis × Kota. A study of the inheritance of rust reaction in a cross between Marquis and Kota to physiologic forms 19 and 27 was previously reported by Hayes and Aamodt (15). Seed of the F_2 plants was still available and used in the present study of the inheritance of reaction to form 1.

In most of the studies already reported on the inheritance of rust reaction in wheat to particular physiologic forms of rust, the parental infection types have been distinct enough so that the various recombinations in the hybrids could be determined with a rather high degree of accuracy.

The infection types produced on Kota and Marquis by form 1, however, while quite distinct, sometimes overlap (34). Kota is moderately resistant and has a "type 3" infection which is described by Stakman and Levine (34) as follows: "Uredinia medium in size; coalescence infrequent; development of rust somewhat subnormal; true hypersensitiveness absent; chlorotic areas, however, may be present." Marquis is very susceptible and has a "type 4" infection which is described as follows: "Uredinia large, numerous and confluent; true hypersensitiveness entirely absent, but chlorosis may be present when cultural conditions are unfavorable."

Seedlings of 62 F_3 lines from the Marquis × Kota cross were inoculated with urediniospores of form 1. As expected, the hybrids were difficult to classify. There were various gradations from the "type 3" to the "type 4" of infection and in some cases combinations of the "type 3" and the "type 4" on the same leaf. The parental varieties used as checks were quite distinct in their infection types and showed very little overlapping. Several

of the F_3 hybrids also appeared to be homozygous for infection type. In order to be certain of the determinations of rust reaction the experiment was repeated by inoculating another set of the same material. The majority of the hybrids showed approximately the same reaction as in the first test. On the basis of the combined results from both tests, the F_3 lines were classified as follows: 16 moderately resistant, 39 heterozygous, and 7 very susceptible. The theoretical expected on the basis of a 1:2:1 ratio would be 15.5:31:15.5. The data are presented in table 18 and fitted to a theoretical 1:2:1 ratio by the goodness of fit method.

TABLE 18.—*Segregation of rust reaction in F_3 of a cross between Marquis and Kota and calculation of a goodness of fit to a 1:2:1 ratio*

Class	F_3 lines		O − C	$(O-C)^2$	$\frac{(O-C)^2}{C}$
	Observed	Calculated			
Susceptible	16	15.5	0.5	0.25	0.016
Heterozygous	39	31.0	8.0	64.00	2.065
Moderately resistant	7	15.5	8.5	72.25	4.661
Total	62	62.0			

P = 0.035 $X^2 = 6.742$

As bad a result, or worse, would be expected on the basis of random sampling in about 4 per cent of such trials. Additional data were made available through a study of the ratios of immune to susceptible plants in the heterozygous F_3 lines.

There were 848 plants in the 39 heterozygous F_3 lines, 501 of which were classed as moderately resistant and 347 as susceptible. In some lines there was a preponderance of the "type 4" of infection, and in others a preponderance of the "type 3," and in still others the two types of infection were about equally frequent. There appeared to be no transgressive segregation in either direction nor types that were consistently intermediate between the two parents.

These results seem to indicate that the factors which govern the reaction of Kota and Marquis to physiologic form 1 are allelomorphic to each other as well as to the factor for immunity in Kanred. The reaction of Marquis, Kota, and Kanred to physiologic form 1 is governed by a series of three multiple allelomorphic factors. The Kanred factor results in an immune reaction, the Kota factor in a moderate resistance, and the Marquis factor in complete susceptibility (Fig. 2).

Host Morphology

The physico-chemical properties of the sap of different wheat varieties have been studied by several investigators and no consistent correlations were found between these properties and rust resistance. Certain internal structures of the host have been shown to determine in some measure the extent and spread of development of the fungus. Extensive studies of the morphology of different wheat varieties and a comprehensive literature review on the subject have been made by Hursh (21). He found that the sclerenchymatous tissue in the stems of certain resistant varieties was very large in proportion to the amount of chlorenchymatous collenchyma. As the rust fungus can live practically only in the collenchyma, it is evident that the amount of collenchyma may possibly limit to a considerable extent the development of rust sori.

Histological examinations were made by the writer, of seedling leaves grown in the greenhouse and stems from plants grown in the field, in an attempt to find some explanation for the different reactions of the plants to rust attacks. Seedling leaves of Marquis and Kota which showed a considerable difference in the size of the rust pustules appeared to have no structural differences which could be correlated with the infection types. Sclerenchymatous tissue was found primarily at the extreme edge of the leaf and next to the vascular bundle of the mid-vein. In both regions the sclerenchyma cells were located just beneath the epidermis.

Seedling plants of the 16 F_3 families homozygous for the "type 3" of infection and the 7 F_3 families homozygous for the "type 4" were again grown in the greenhouse and inoculated with physiologic form 1. The reactions of the plants to the pathogen were in agreement with the first two trials. These leaves were then examined histologically and found to show no outstanding structural differences which might be correlated with the two infection types. The shape of the epidermal cells and the thickness of the cell walls appeared to be associated with infection types in a few cases but they were not consistently so. Likewise, it did not seem that the limited production of sclerenchymatous tissue in the seedling leaves could in any way, at least mechanically, have an influence on the development of the rust sori.

Transverse sections of Marquis and Kota stems from field-grown material were examined histologically and found to show approximately the same structural relationships as reported by Hursh. The collenchymatous areas are confined to regions within the sclerenchymatous tissue. In Kota these areas are small and not continuous. In Marquis the collenchymatous areas are larger and often several were confluent and formed a continuous area of collenchyma. Such coalesced areas were not so numerous in this

variety for the seasonal condition under which the experiment was conducted as in the case reported by Hursh (21).

Stems of the F_3 hybrid lines were examined in this same manner. There appeared to be no apparent differences in the ratios of sclerenchyma to collenchyma between the hybrids which showed a "type 3" infection and those which developed a "type 4." All of the hybrids examined showed development of sclerenchyma intermediate between that of the two parental varieties. When these plants were grown in the field under an artificial epidemic of stem rust, produced by spraying with a large number of different physiologic forms, there were no observable differences in their rust reactions.

Kanred stems also were examined for their structural characteristics. A larger number of the collenchyma areas were confluent in it than in Marquis, and they appeared to be about as in the Marquis described by Hursh. Two Marquis × Kanred hybrids also were examined for their sclerenchyma-collenchyma ratio. One of these selections was susceptible to form 1, like Marquis, and the other was immune, like Kanred. Both of the hybrids had the same structural characteristics, however, and were quite similar to Marquis.

The histological studies on the structural differences in the seedling leaves and the stems of Marquis, Kota, Kanred, and several hybrids fail to demonstrate any particular relationship between these differences and the reactions of these same varieties and selections to physiologic form 1.

DISCUSSION

The nature of the processes involved in bringing about the heading period of our winter wheats has been a matter of speculation and experiment for some time. The environmental conditions which control these processes and permit winter grains to head and to produce seed are closely interrelated with each other. Some investigators believe that both spring and winter wheats undergo a pause in the course of development and that it is short in the case of spring wheats and long in the case of winter wheats. This is probably true when the environmental conditions are such that growth is more favorable for the spring wheats than it is for the winter wheats. The climatic conditions in the field and greenhouse in the spring of the year appear to favor the spring wheat more than the winter wheats in this respect. The results obtained by the writer with Marquis, Kota, and Kanred and those obtained by Klages with several other varieties seem to demonstrate this fact.

On the other hand, these same varieties showed no great differences in the form of their growth curves when planted in the fall in the greenhouse. The

writer's results are in agreement with those of Klages, and Maximov and Poiarkova in this respect. The conditions for growth in the greenhouse in the fall of the year do not seem to favor the spring wheats any more than the winter wheats. Both wheats respond equally well and show a continuous growth curve with no indication of a period of dormancy. Low temperatures were not necessary to force the winter wheats into continuous growth until flowering.

Winter wheats, when spring-sown, have a tendency to fall into a resting period. Freezing temperatures, however, seem to release the necessary stimulus for a continuous growth curve. Kanred grown from spring plantings in the greenhouse will have a continuous growth curve if transplanted to the field early enough to be subjected to low temperatures, while it remains dormant if left in the greenhouse or transplanted to the field after the freezing temperatures have passed. These results appear to be in agreement with the observations made by Call and Salmon, and by Jensen, that winter wheat will not go into a dormancy period if it is planted early enough in the late winter or early spring. The results indicate that length of day and temperature are both important factors in this complex process. It is quite evident that these variations from the usually observed course of development are probably the result of changes in environment which allow the inherited potentialities of the plants to come to expression. Under low intensities of light, as from fall sowing in the greenhouse, there appears to be little difference in the form of growth curves of spring and winter wheats. Under the higher light intensities and longer daily light duration of the early spring, the spring wheats have a normal growth curve and the winter wheats remain for some time in the vegetative stage. In this latter case it appears as though low temperatures may take the place of light and furnish the necessary stimulus so that winter wheat may produce heads.

After a consideration of the influence of the environmental conditions upon the development of such characters as growth habit and time of heading, it is evident that the data can not be analyzed and interpreted with as much precision as may be desired. The results show that there is a rather complicated relationship in the genetic differences between spring and winter wheats. That several factors are concerned with the inheritance of this character appears to be a common opinion. The influence of the environmental conditions upon the plant's ability to produce heads, without a dormancy period intervening, is sufficient to explain the small differences in results obtained from year to year or place to place. Gaines concluded that he had a rather high proportion of spring to winter types, compared to other investigators, owing to the early planting and the fact that the conditions were such that the winter parent plant headed. A winter wheat grown from spring-sown seed in a region where the days are long is more likely to head

than in a region where the days are short. The influence of such varied climatic conditions upon the growth habit of a wheat demonstrates the impracticability of completely correlating the results of different investigators. The variation in results reported may also be accounted for by wide genetic differences which various wheat varieties may have for this character. The segregation which one would expect in a cross depends upon the factors present for growth habit in the parental material, date of seeding, and the environmental conditions peculiar to the season in which the hybrid plants are grown.

In the cross between Marquis and Kanred, the breeding behavior of the F_2 and F_3 plants indicated that there is a two-factor difference for growth habit between these two varieties. The same relationship holds also for the cross between Kota and Kanred. Marquis and Kota each contain two pairs of factors for spring habit which are dominant to the recessive allelomorphs in Kanred for winter habit. The F_2 produce an approximate 15:1 ratio for spring and winter types. The factorial hypothesis used to explain these results was corroborated as being the most plausible explanation as shown by the breeding behavior of the F_3.

The data also seemed to indicate that there are at least two factors for early and late heading in addition to the two factors for growth habit. The results are not as conclusive for this character as for growth habit, and further study will be necessary before much certainty can be attached to the nature and number of the factors concerned.

It was suggested that the genotypic constitution of the late-heading Marquis × Kanred hybrid would be AA bb cc dd. When it is crossed with Marquis, which is probably AA BB CC DD, the F_1 will be AA Bb Cc Dd. It would be an early spring type. No winter forms would be expected in the later segregating generations, as both parents are homozygous for the A factor. There would be a segregation in the F_2 for early and late heading types in an approximate 15:1 ratio. The actual results obtained from this cross fairly well fitted the theoretical expectations according to the above hypothesis.

In order to prove more conclusively the factorial constitution of these late spring types and the hypothesis in general, a study of the type of segregation in intercrosses between several late-heading spring types and with the winter parent would probably throw considerable light on the problem. Two late spring selections, each one containing a different pair of the factors for spring habit, and the reciprocal pair of recessive factors for winter habit, when crossed, ought to produce some winter types in the F_2. The crosses would be as follows: AA bb × aa BB. The F_1 factorial composition would be Aa Bb and the plant a spring type. One plant out of every

16 F_2 segregates would contain the recessive factors for winter habit, aa bb. Crosses of this type are contemplated, and theoretically it seems should demonstrate that some plants with winter growth habit can be produced by a cross between two late spring wheats.

In an inheritance study of rust reaction in crosses between Marquis and Kanred it was definitely demonstrated that immunity from several forms of stem rust is governed by a single genetic factor. The present studies on 54 F_7 lines from this cross further substantiated the belief that such was the case. Three of these lines appeared to be a mixture of types for rust reaction due to an error in the first determination in the F_3. The other 51 lines, however, were practically homozygous for the immune reaction determined previously.

The studies of the inheritance of reaction of seedlings to physiologic form 1 in crosses between Kota and Kanred indicate that the factor for moderate resistance in Kota is also allelomorphic to the factor for immunity in Kanred. Immunity is dominant to moderate resistance and the ratios approximated very closely that of a monohybrid. The factor in Marquis for susceptibility and in Kota for moderate resistance to the same rust form are both allelomorphic to the factor for immunity in Kanred and probably are therefore allelomorphic to each other. Further evidence to this effect was obtained by studying the reaction of the F_3 plants of a cross between Kota and Marquis to this particular physiologic form of rust. These results indicated also that the factors for reaction to form 1 in Kota and Marquis are allelomorphic to each other.

A histological study of certain morphological characteristics of these varieties failed to show structural differences which were correlated with any of the three reaction types and their genetic factors. The basic differences for rust reaction appeared to be primarily physiological. There were wide differences in the proportional amounts of sclerenchyma and collenchyma in the different varieties and hybrids, but there was no close correlation between such structures and their seedling reaction to form 1 in the greenhouse and the reaction to this and other forms in the field.

SUMMARY

1. Kanred winter wheat, when sown in the greenhouse in the fall of the year, shows a continuous growth curve similar to that of spring wheats, with no indication of a period of dormancy. Freezing temperatures are not essential to culm and flower formation.

2. Kanred, when sown in the greenhouse in the late winter or early spring, will remain dormant for several months and not show a continuous growth curve as do the spring wheats. Freezing temperatures in this case act as a necessary stimulus which causes winter wheat to head.

3. There were 4,811 spring types to 442 winter types in the F_2 of the cross between Marquis and Kanred; and 1,177 spring types to 128 winter types in the cross between Kota and Kanred. In the F_3 the ratio approximated 7 lines homozygous for spring habit to 8 heterozygous for spring and winter habit in material in which the winter types had been eliminated in the F_2. In the F_3, from a random F_2, the ratio approximated 7 pure spring to 8 heterozygous to 1 pure winter. Approximately one-half of the heterozygous lines segregated into 15:1 ratios for spring and winter types and the other half into 3:1 ratios.

The results were explained by the hypothesis that Marquis and Kota each contain two pairs of dominant factors for spring habit and Kanred contains the recessive allelomorphs for winter habit.

4. There are several factors concerned with early and late heading in addition to the two factors for growth habit. Earliness was dominant to lateness. The segregation in the F_2 and F_3 was too complex to permit a detailed genetic analysis of the factors concerned with this character. Further studies are necessary before more definite conclusions can be drawn.

5. A backcross of a late spring hybrid selection from Marquis × Kanred to Marquis failed to produce winter types which typified Kanred in the segregating generations. The segregation for early and late heading suggested at least a two-factor difference with earliness dominant to lateness.

6. Seedling posture shows a general correlation with growth habit. This relationship is only relative and can not be taken as an absolute criterion in the selection of winter types.

7. The immunity of Kanred from several physiologic forms of stem rust in crosses between Marquis and Kanred is governed by a single genetic factor.

8. Immunity is dominant to susceptibility in crosses between Marquis by Kanred and Kota by Kanred to physiologic form 1. The factors for susceptibility to physiologic form 1 in Marquis and for moderate resistance in Kota are both allelomorphic to the factor for immunity in Kanred.

9. A study of F_3 plants from a cross between Marquis and Kota indicated that factors for the reactions of these two varieties to form 1 are also allelomorphic to each other. From these results it was concluded that there is a multiple allelomorphic series of three factors which governs the reaction of Marquis, Kota, and Kanred, to physiologic form 1.

10. No consistent correlations were found between the reaction to form 1 and the structural differences of the parents or the hybrids. The differences in reaction to this form of rust to which Marquis is susceptible and Kota is moderately resistant appear to be due to physiologic causes.

11. The inheritance of growth habit and rust reaction are in accord with Mendelian laws.

BUREAU OF PLANT INDUSTRY,
UNITED STATES DEPARTMENT OF AGRICULTURE,
IN COOPERATION WITH
MINNESOTA AGRICULTURAL EXPERIMENT STATION,
ST. PAUL, MINN.

LITERATURE CITED

1. AAMODT, O. S. The inheritance of growth habit and resistance to stem rust in a cross between two varieties of common wheat. Jour. Agr. Res. **24**: 457–470. 1923.
2. AKERMAN, A. Resistance shown by numerous varieties of wheat at Svalöf during the winter of 1921–22. Int. Rev. Sci. and Pract. Agr. n. s. **1**: 350–353. 1923.
3. ANONYMOUS. Agricultural statistics. U. S. Dept. Agr. Yearbook **1925**: 741–1514. 1926.
4. CALL, L. E. and S. C. SALMON. Growing wheat in Kansas. Kan. Agr. Exp. Sta. Bul. 219. 1918.
5. CAPORN, A. ST. CLAIR. An account of an experiment to determine the heredity of early and late ripening in oats. Jour. Genetics **7**: 247–257. 1918.
6. CLARK, J. A. Cereal experiments at Dickinson, N. Dak. U. S. Dept. Agr. Bul. 33. 1914.
7. ———, J. H. MARTIN and C. R. BALL. Classification of American wheat varieties. U. S. Dept. Agr. Bul. 1074. 1922.
8. ———, ——— and J. H. PARKER. Comparative hardiness of winter-wheat varieties. U. S. Dept. Agr. Circ. 378. 1926.
9. COOPER, H. P. The inheritance between typical spring and winter growing habits in crosses between typical spring and winter wheats, and the response of wheat plants to artificial light. Jour. Amer. Soc. Agron. **15**: 15–25. 1923.
10. FRUWIRTH, C. Allgemeine Züchtungslehre der landwirtschaftlichen Kulturpflanzen. (Handbuch der landwirtschaftlichen Pflanzenzücht. Bd. 1). Berlin. 1910.
11. ———. Die Umzüchtung von Wintergetreide in Sommergetreide. Ztschr. Pflanzenzüchtung **6**: 1–46. 1918.
12. GAINES, E. F. Inheritance in wheat, barley and oat hybrids. Wash. Agr. Exp. Sta. Bul. 135. 1917.
13. ——— and H. P. SINGLETON. Genetics of Marquis × Turkey wheat in respect to bunt resistance, winter habit and awnlessness. Jour. Agr. Res. **32**: 165–181. 1926.
14. HARRINGTON, J. B. and O. S. AAMODT. Mode of inheritance of resistance to *Puccinia graminis* with relation to seed color in crosses between varieties of durum wheat. Jour. Agr. Res. **24**: 979–996. 1923.
15. HAYES, H. K. and O. S. AAMODT. A study of rust resistance in a cross between Marquis and Kota wheats. Jour. Agr. Res. **24**: 997–1012. 1923.
16. ——— and R. J. GARBER. Breeding small grains in Minnesota. Minn. Agr. Exp. Sta. Bul. 182. 1919.
17. ——— ———. Breeding crop plants. 328 pp. McGraw-Hill Book Co., New York. 1921.

18. ————, J. H. PARKER and C. KURTZWEIL. Genetics of rust resistance in crosses of varieties of *Triticum vulgare* with varieties of *T. durum* and *T. dicoccum*. Jour. Agr. Res. **19**: 523–542. 1920.
19. ———— and E. C. STAKMAN. Wheat stem rust from the standpoint of plant breeding. Proc. Ann. Meeting West. Canad. Soc. Agron. **2**: 22–35. 1922.
20. ————, ———— and O. S. AAMODT. Inheritance in wheat of resistance to black stem rust. Phytopath. **15**: 372–387. 1925.
21. HURSH, C. R. Morphological and physiological studies on the resistance of wheat to *Puccinia graminis tritici*, Eriks. and Henn. Jour. Agr. Res. **27**: 381–412. 1924.
22. JENSEN, I. J. Winter wheat studies in Montana with special reference to winter killing. Jour. Amer. Soc. Agron. **17**: 630–631. 1925.
23. KILLER, J. Über die Umzüchtung reiner Linien von Winterweizen in Sommerweizen. Jour. Landw. **67**: 59–62. 1919.
24. KLAGES, K. H. Metrical attributes and the physiology of hardy varieties of winter wheat. Jour. Amer. Soc. Agron. **18**: 529–566. 1926.
25. KOERNICKE, F. Arten und Varietäten des Getreides. 370 pp. Berlin. 1885.
26. LINNAEUS, C. Species plantarum. 1753.
27. MAXIMOV, N. A. and A. I. POIARKOVA. The physiological nature of winter and spring forms of cereals. Bul. Applied Bot. and Plant-breed. **14**: 211–234. 1925. In Russian. Abst. in Bot. Abs. **15**: 15. 1926.
28. MARTIN, J. H. Comparative studies of hardiness in wheat. Jour. Agr. Res. In press.
29. NILSSON-EHLE, H. Zur Kenntniss der Erblichkeitsverhältnisse der Eigenschaft Winterfestigkeit beim Weizen. Ztschr. Pflanzenzücht. **1**: 3–12. 1912. Abst. in Exp. Sta. Rec. **29**: 635–636. 1913.
30. PUTTICK, G. F. The reaction of the F_2 generation of a cross between a common and a durum wheat to two biologic forms of *Puccinia graminis*. Phytopath. **16**: 205–213. 1921.
31. SALMON, S. C. Why cereals winterkill. Jour. Amer. Soc. Agron. **9**: 353–380. 1917.
32. SCHAFER, E. G. Inheritance studies. Wash. Agr. Exp. Sta. Bul. 180. 1923.
33. SPILLMAN, W. J. Application of some of the principles of heredity to plant breeding. U. S. Dept. Agr., Bur. Plant Indus. Bul. 165. 1909.
34. STAKMAN, E. C. and M. N. LEVINE. The determination of biologic forms of *Puccinia graminis* on Triticum spp. Minn. Agr. Exp. Sta. Tech. Bul. 8. 1922.
35. TAKAHASHI, N. Studies on the inheritance of the spring and winter growing habit in crosses between spring and winter barleys. Bul. Chosen Agr. Exp. Sta. **2**: 1–7. 1925.
36. VASSAR, L. P. Field and laboratory methods for a determination of winter hardiness in winter wheat hybrids. Unpublished thesis, Univ. of Minn. June, 1924.
37. VAVILOV, N. I. and E. S. KUZNETSOVA. The genetic nature of winter and spring varieties of plants. Izvestii Agronomicheskogo Fakul'teta Saratovskogo Univ. (Bul. Agron. Faculty Saratov Univ.) **1**: 25. 1921. In Russian. English summary, pp. 23–25.
38. WALDRON, L. R. Value of continuous selection and its bearing upon hardiness in winter wheat. Ann. Rept. Amer. Breeders' Assoc. **7**: 74–79. 1912.

BACTERIAL BLIGHT OF PEA: OVERWINTERING, DISSEMINATION, AND PATHOLOGICAL HISTOLOGY

VLADIMIR SKORIC[1]

INTRODUCTION

Bacterial blight of peas was first described by Sackett (5) in Colorado in 1915. It was later noted by Jennison (2) in Montana. Ludwig (4) reported the occurrence of the disease in South Carolina, and it has recently been found by Jones and Linford (3) to be prevalent in pea fields of Wisconsin. From these reports it is clear that the disease is widespread in pea districts, causing more or less severe damage. The symptoms of the disease were described in detail by Sackett and by Ludwig. The possibility of seed transmission was mentioned by Jennison, and a more definite indication that the bacteria overwinter in the form of a dried film on the surface of the seed was given by Jones and Linford. Sackett gives a brief account of the histology of the diseased tissue, in which he mentions the penetration of the organism through wounds and stomata into the stem and leaves and further spread into the underlying parenchyma. According to the findings of the last-mentioned author, the infection does not appear to extend into the pith, stereome, and vascular bundles, although wilting of petioles was not uncommon. He observed a gradual wilting of plants when artificially inoculated, but not a sudden collapse, and therefore he was inclined to consider it a withering rather than a wilting. The present investigation was undertaken in order to determine whether the organism is seed-borne, and to make observations on the method of dissemination. A study was also made of the pathological histology of diseased plants and the ability of the parasite to attack other leguminous plants.

SYMPTOMS OF THE DISEASE

Although Sackett and Ludwig have given detailed accounts of the symptoms of the disease, some additional observations have been made.

When flowers and pods were sprayed with water suspensions of the bacteria, stomatal infection was repeatedly found to occur on the sepals and

[1] This research was carried out at the Department of Plant Pathology, University of Wisconsin, under the direction of Professor L. R. Jones, whom the writer sincerely thanks for helpful criticism and advice. Special acknowledgments are due Professor W. H. Wright for help in cultural work and to Dr. F. R. Jones and Dr. M. B. Linford for helpful suggestions and cooperation during this work.

bracts on the peduncle, the disease later spreading into the stem and pods. The flower-buds were often killed before they could open. When invasion of sepals occurs at the time of pod formation, these soon shrivel and decay. If pods are further developed, the disease spreads to them, causing lesions of various size and cracking of the pods, but the seed is not usually prevented from ripening. When the bacterial invasion on such pods was largely along the dorsal suture (Plate XXIII), the funiculus and surface of some seeds was very often found enveloped in bacterial slime. The funiculus was in this case often deep green and water-soaked. In many instances the seed showed green water-soaked spots only near the one or the other end of the hilum.

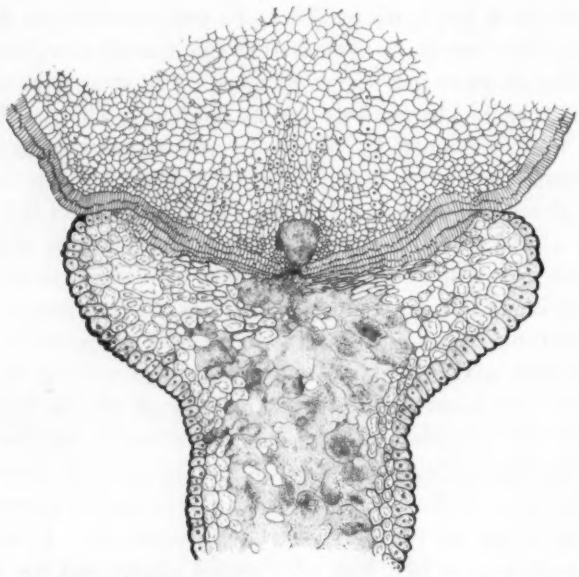


FIG. 1.—Longitudinal section of the funiculus and lower part of the seed showing penetration of bacteria through the micropyle into the seed coat (×105).

On inoculating the stems of pea plants with needle pricks, the observation was made that many plants so infected showed wilting of leaflets. The larger lesions occurred at the bases of stipules and leaflets, and the whole plant wilted after eight to ten days.

If infected plants were kept in moist chambers for 10 to 20 hours, a grayish-white bacterial ooze in the form of droplets (Plate XXII, B) was observed, which sometimes later became brownish as described by Sackett. Closer observation of infected plants at short intervals revealed the fact that the bacteria extrude from the diseased plant at first in the form of short cirrhi (Plate XXII, A) and only later, by absorption of water, form drop-

lets of slime containing bacteria. Professor L. R. Jones has stated to the writer that he has observed this formation of cirrhi under field conditions to be so abundant on the pod as to simulate the appearance of downy mildew.

ISOLATION AND IDENTIFICATION OF THE CAUSAL ORGANISM

The organism used in these studies was isolated at different times, from living plants and from the surface and inner part of the seed, from the funiculus, and from badly spotted pods, all gathered in July, 1926, at the University Hill Farm near Madison, Wisconsin. Twelve strains from several isolations made during the winter of 1926 and spring of 1927 were grown on the following media: nutrient broth, meat infusion broth, nutrient agar, glucose agar, potato dextrose agar, plain gelatin, glucose gelatin, starch agar, sodium caseinate agar, plain milk agar, plain milk, litmus milk,

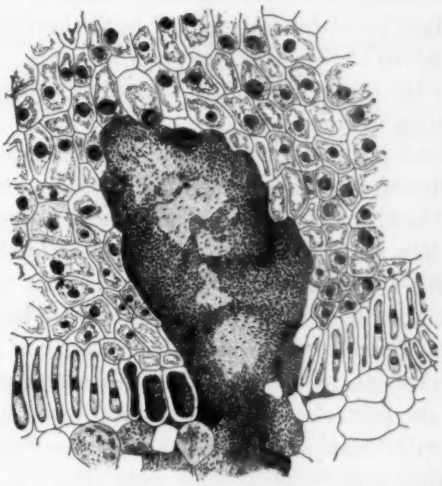


FIG. 2.—A detail from figure 1 showing bacteria in micropylar opening and in the cavity in seed coat (×1,000).

lactose broth, glucose broth, sucrose broth, Buchanan's solution; peptone medium using Difco, Witte's, and Parke & Davis peptone for methyl red and Voges-Proskauer test, Fermi's solution, Uschinsky's solution with corrected reaction to pH 7.0 and with uncorrected reaction, and finally in Cohn's solution.

The behavior of all the strains of the organism on culture media, which were identical with those used by Sackett, was in all cases the same excepting slight differences in plain and litmus milk, where soft curd production was not observed. The cultivation of the organism on starch agar has given in all twelve strains distinct hydrolysis of starch below, and to a certain

extent around, the giant-colonies. The organism grew very abundantly and rapidly in Buchanan's solution and in different peptone media, giving a negative test in both methyl red and Voges-Proskauer test. In spite of the insignificant cultural differences in plain and litmus milk, and in spite of the hydrolysis of starch—which may have been due to the differences in the method used by Sackett and that used in this investigation, we consider that the organism with which we have been working is identical with *Pseudomonas pisi* Sackett (*Phytomonas pisi* (Sackett) S. A. B.).

OVERWINTERING AND SEED TRANSMISSION OF THE ORGANISM

As has been mentioned previously, there are suggestions in the literature that the organism may be seed-borne. To solve this problem, trials were made to isolate the organism from the seed. The seed used for this purpose were in closed pods which had been carefully collected in June, 1926. The pods showed abundant bacterial lesions and were therefore considered very suitable for the isolation of the organism from the seed. The pods were air-dried and stored in the laboratory until November, when the first attempts to isolate the organism were made.

The surface of the pods which were found to be completely closed was disinfected by treatment for five minutes in mercuric chloride and washed in sterile water. Then the pod was opened under aseptic conditions in a sterile petri dish. The seeds were transferred with sterile forceps to sterile water blanks, which were shaken thoroughly from time to time and allowed to stand for half an hour to permit the distribution of bacteria in the water. The plates poured from the water used in these first attempts remained sterile. Since it was suspected that the time in water was too short to allow the distribution of the bacteria, in the next isolations the seed were allowed to stand in sterile water blanks over night and plates were poured from this suspension. This time numerous colonies were obtained on potato dextrose agar quite like the colonies of *Pseudomonas pisi*, and later inoculations have shown that the organism thus obtained is pathogenic to pea plants, producing characteristic lesions on the stems and abundant stomatal infection on stipules and leaves.

Shortly thereafter, it was found by closer examination of the seed from infected pods that white, dry films are present on their surfaces. When seed with such films were used for isolation, the development of the organism on plates was very abundant, and in most cases a pure culture of *Pseudomonas pisi* was obtained. To establish whether this film contains bacteria, a small piece of the film was transferred to a sterile water drop on a sterile slide, crushed, and allowed to spread and dry. After fixing and staining with carbol fuchsin, it was possible to see that the whole microscopic field

contained shortened rods, sometimes almost cocci-like, embedded in slime. To prove that the bacteria contained in the dry film were alive and identical with the causal organism of bacterial blight, an attempt was made to plate out a small part of the bacterial film. About 3 square millimeters of film were taken with a sterile scalpel, transferred, and crushed in a water drop in a sterile petri dish. After a short time, when it was considered that the organism was properly distributed in the drop, one loopful was transferred to each of a second and third petri dish. Plates were poured with potato dextrose agar, and after 36 hours bacteria developed very abundantly in the first plate, a few colonies in the second, and none in the third. That these were pure cultures of the pea blight organism was shown by examination and confirmed by the production of infection on inoculated plants. Further observations have shown that, although the film may be invisible to the naked eye, its presence can be demonstrated by isolation of the organism from the

TABLE 1.—*The number of bacteria present on the surface of seed from pea pods infected with bacterial blight*

Seed no.	Isolation date, 1927	No. of dilution plates	Average no. of colonies[a]	Inoculation results[b]	Check
1	Jan. 5	3	3540	+	Healthy
2	do	3	0		
3	do	3	94	+	Healthy
4	do	3	20	+	do
5	Feb. 8	3	30	+	do
6	do	3	0		
7	Mar. 2	5	15400	+	Healthy
8	do	3	0		
9	do	3	380	+	Healthy
10	Apr. 6	3	70	+	do
11	do	3	623	+	do
12	do	3	0		
13	May 12	4	1840	+	Healthy
14	do	3	0		
15	June 2	5	yellow saprophyte		
16	do	5	do		
17	do	3	0		
18	do	5	21200	+	Healthy
19	do	3	1500	+	do
20	do	3	40	+	do
21	do	3	0		

[a] In the cases where the number of the colonies was large, a conspicuous bacterial film was always present.

[b] + = Abundant infection.

surface of the seed. Several attempts were made to determine the number of viable bacteria on the surface of seed. The seed were placed in sterile water blanks and kept over night at 4° C. to permit the diffusion of the organism in the water, the temperature being low enough to prevent their multiplication. According to the cloudiness of the suspension, three to five dilution plates were poured, the colonies counted, and the pathogenicity established by inoculations. In table 1 are given averages of plate-counts showing the number of viable bacteria on individual seed. The results are variable, as would be expected, considering the differences in the severity of pod infection.

In several cases it was noted that seed having no visible trace of bacterial film on the surface nevertheless gave rise to milky clouds when the water blanks in which they had been soaked for some time were shaken. This indicated that there might be a possibility of the overwintering of the organism other than on the surface of the seed. When such seed were thoroughly

TABLE 2.—*Isolations of Pseudomonas pisi from pea seed over a period of 10 months after collection*

Date of isolation	No. of seed examined	No. of seed containing organism	Manner of inoculation	Result of inoculation[a]	Remarks
Nov. 10, 1926	10	6	Needle-pricks and spraying with bacterial suspension	+	Abundant stomatal infection and stem lesions
Dec. 9, 1926	10	4	do	+	do
Jan. 3, 1927	8	3	do	+	Stem, pods, sepals, and leaves abundantly infected
Feb. 5, 1927	8	4	Sprayed with bacterial suspension	+	Stomatal infection on sepals and leaves
Mar. 5, 1927	2	2	do	+	Seeds with water-soaked spots
Apr. 6, 1927	4	3	do	+	do
May 12, 1927	5	3	do	+	Two seeds with water-soaked spots
June 2, 1927	10	6	do	+	Three seeds with water-soaked spots

[a] + = abundant infection.

examined, a water-soaked spot beside the hilum was discovered (Plate XXI, A). Water-soaked lesions completely surrounding the hilum were seldom found.

To test whether this discoloration is a local change in color so often found on dry pea seed or a water-soaking caused by the penetration of the organism, some seed showing these symptoms were disinfected for five minutes in mercuric chloride and washed in sterile water. The seed were allowed to stand overnight in sterile water and plates were poured from this water. Positive inoculations with the organism isolated from such seed confirmed its identity with *Pseudomonas pisi*. The isolations were repeated several times with the same success.

The abundance of the organism in a dormant condition, but alive and virulent on the surface and within the seed during a period of 10 months is shown in table 2.

The percentage of seed with clearly visible films and with water-soaked spots in several lots taken from heavily infected pods was determined. The average of counts gave 13 per cent of seed with the bacterial film on the surface and 2.5 per cent with lesions.

The next step was to establish whether the disease can be transmitted by infected seed to the young plant. For this purpose, seed which showed water-soaked spots and which came from underneath lesions on the pods were selected. The seed bearing water-soaked spots were considered to be internally infected, and those from below pod lesions to be carrying the organism on the surface. The seed with internal infection were disinfected with mercuric chloride for five minutes, and the seed with the organism on the surface were not treated. As a control, seed from healthy pods of the same variety were sown in sterile soil and pots. The result of two sowings are shown in table 3.

TABLE 3.—*The development of blighted pea plants from infected seed and the effect of soil moisture on the development*

Date of sowing, 1927	Pot no.	No. seed	Location of organism	Treated with	No. of diseased plants	Remarks
Feb. 1	1–6	33	In seed	$HgCl_2$	6	Abundantly watered
do	7–29	136	On seed	.....	9	do
do	30–34	40	Control	.....	0	do
Feb. 28	1–3	18	In seed	$HgCl_2$	0	Scantily watered
do	4–6	16	On seed	.....	0	do
do	7–8	16	Control	.....	0	do

The sowing of infected seed has shown that the disease can be transmitted by this means, but at the same time the importance of the water content of the soil in the transmission of the disease has been indicated.

The appearance of the symptoms on diseased plants was very interesting, suggesting that the organism usually does not penetrate into the cotyledons and embryo in the seed, but that penetration takes place through the surface of the developing seedling. Most of the diseased plants, developed from infected seed, showed the infection only on the lowest pair of stipules. Some of them showed symptoms on the first, second, and third stipules, and first and second pair of leaflets. All organs of a few plants became infected, especially the apices, whence the disease spread downward and killed the

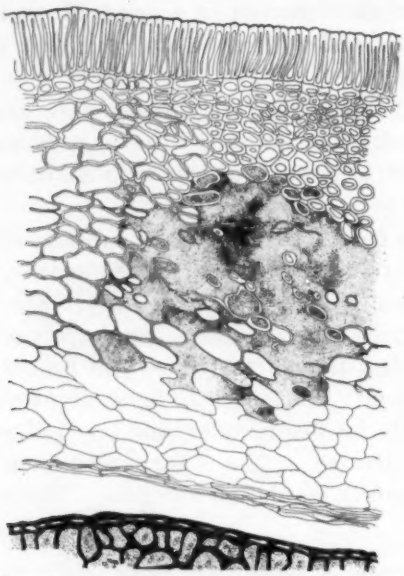


FIG. 3.—Cross-section of seed coat of ripe seed showing the location of bacteria (×460).

young plants. After the development of symptoms on such plants, it was possible to find that the position of many of the lesions corresponded to that of the lesions on the stipules which were covering them during the emergence of the plant from the soil. This distribution of lesions indicates that the outer covering of the plumule becomes infected by touching the infected seed coat and transmits the disease to some or all the organs beneath it according to the rapidity or slowness of the development of the young plant. This is a similar method of infection to that described by Jones and Linford (3) in *Ascochyta pisi*.

The severe outbreaks of the disease in pea fields in previous years have suggested to several observers that the organism may survive in the soil.

A few attempts by the writer to determine whether or not the organism was present in soil on which diseased peas had been found during several previous years have given negative results.

FIELD OBSERVATIONS ON INCIDENCE AND DISSEMINATION OF THE DISEASE

As it had been shown that the organism is viable on and in the seed, and that from such seed diseased plants developed in the greenhouse, a study was also made of the development of the disease under field conditions. For this purpose several pea fields near Columbus, Wisconsin, were visited, and especial consideration was given to trial plots of E. J. Renard at the University Hill Farm near Madison, Wisconsin. When the pea plants were still quite small (about 10 to 12 cm. high), it was not difficult to distinguish plants which showed the disease in a form characteristic for plants infected

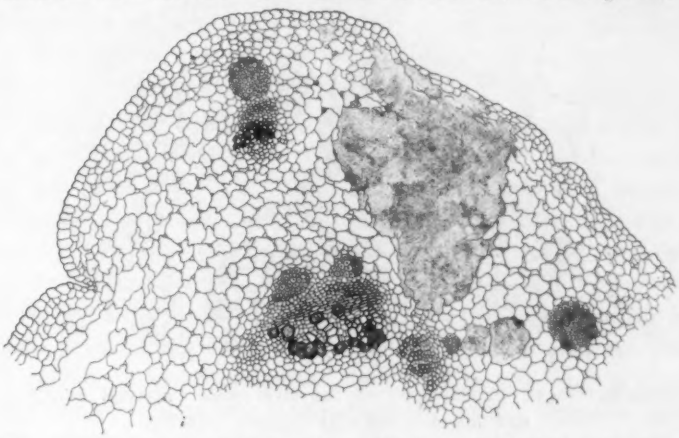


FIG. 4.—Cross-section of a part of the stem with large bacterial cavity in cortex and vascular invasion of leaf-trace and of vascular bundle (×105).

from seed (Plate XXI, B). The lower stipules and leaves were at this time more or less covered with old lesions of bacterial blight, and there was also secondary infection in the upper part of the plants strikingly like the symptoms of diseased plants which developed from infected seed in the greenhouse experiments. Around such plants were found several plants showing only secondary infection. These plants with secondary infection were found in most cases in the same drill-row and sometimes in the next row when the rows were close together. In the fields where the plants were older, the origin of the disease from one infection center was not so evident. Especially instructive in this regard were the trial plots of the Leonard Seed Company at Columbus, Wis., where Alaska peas from different sources were planted in a field which had not grown peas before. Among many rows

sown at a distance of 75 centimeters apart, each containing about a hundred plants, there were found only three plants apparently infected from the seed and situated in three different rows. Here again the plants around these infected individuals showed only secondary infection. Fortunately, 400 seed remained of the sample which had been used to plant one of the plots in which a diseased plant was found. Among these 400 seed two were found with water-soaked lesions, further evidence that the diseased plants developed from infected seed.

The trial plots at the University Hill Farm also have confirmed previously mentioned findings. All the plots were examined thoroughly for blighted plants: many of them did not show any primary-infected plants; others showed one to nine of such plants in a thousand. Both diseased and

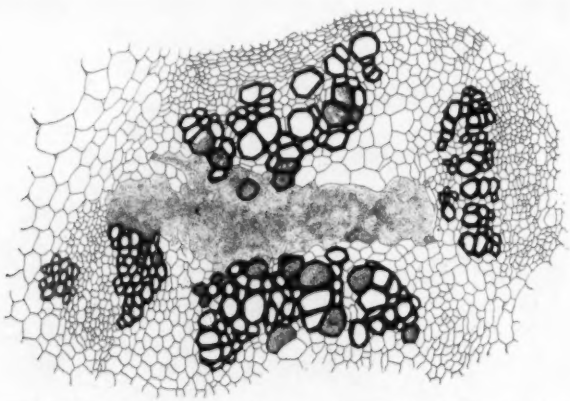


FIG. 5.—Cross-section of central part of the stem with bacterial cavity in the pith and invaded vascular bundles (×460).

healthy plants were grown from seeds from almost every state represented in the trial. Individual examinations of plants in this pea field have shown that in many rows there were plants showing a small amount of secondary infection, although they were not near primary-infected plants. It was noticed that such plants occurred mostly in the lower parts of the field. These infections may be explained by the distribution of the organism in drainage water, as has been shown by Carsner (1) to be the case with angular-leaf spot of cucumber.

PATHOLOGICAL HISTOLOGY

The regular occurrence of water-soaked lesions on one or the other side of the hilum, and nowhere else on the seed surface, suggested that there must be a definite method of migration of the organism into the seed. To trace this migration, pod inoculations were made in two ways: by spraying

with water suspensions of the bacterium; and by introducing the organism by needle pricks into the cavity of the pod, taking care to avoid wounding the seed. Both methods have led to production of water-soaked lesions on the seeds, but again only near the hilum. At the same time more or less extended water-soaking of the funiculus was observed. Young seed with heavily infected funiculi did not develop further; many of them collapsed and dried up. Infected pods and seed were fixed in formal-acetic alcohol, imbedded, and sectioned in the usual way. Sections were stained in weak safranin or carbol fuchsin, Rose Bengal (counter-stained with light green in clove oil), or Giemsa stain, and counter-stained with two per cent safranin in alcohol. The sections show that the tissue of the infected pods was largely invaded by the bacteria, which were abundant in the cells and interstitial spaces. Bacteria penetrated through the wall of the pod, accumulating on the inner surface, on the funiculus, and on the seed, without being able to penetrate into the seed or funiculus. When the bacteria occupied the larger part of the tissue beneath the dorsal suture, they penetrated into the tissue of the funiculus and migrated toward the seed. In some cases the bacteria destroyed so much of the tissue of the lower part of the funiculus that the seed underwent no further development. In other cases the organism traversed the funiculus to the micropylar opening, and through this natural opening entered the integuments (Figs. 1 and 2), preventing the development of the young seed. If the seed was sufficiently developed when the bacteria reached the micropylar opening, they entered into the seed coat and the seed ripened normally. In such cases, if the bacteria penetrated in large numbers, water-soaking near the hilum resulted, but a slight penetration may occur without any macroscopically visible signs on the surface of the seed. Sections were made of mature seed collected in 1926 and also of seed nearly mature with water-soaked lesions from artificial inoculations in the greenhouse. Also seed from field material collected in June, 1926, was examined for the same purpose. In both cases, it was possible to demonstrate that the bacteria are confined to the seed coat. They were either intercellular or intracellular in position and produced smaller or larger cavities filled with bacteria embedded in slime (Fig. 3). It was not possible to observe whether the bacteria penetrated the cotyledons, but in some instances they were found occupying the cells of the seed coat in close proximity to the rootlet of the embryo. Therefore it seems probable that sometimes they may enter the young embryo. Such infection may explain that fact that the more heavily infected seed fails to develop at all or dies soon after producing only a small rootlet. The organism has been isolated from seed and seedlings killed in this manner.

Artificial inoculations of the stem by needle pricks have in many instances produced wilting of leaflets and of whole plants, but some plants

which have developed from infected seed also wilted. Therefore it was assumed that the bacteria entered the vascular bundles and caused the wilting by plugging the vessels. The examination of slides obtained from such wilted plants has shown the vessels filled with bacteria (Figs. 6 and 7). Since all the vessels are not completely plugged with bacteria, it is probable that the abundant slime produced by them is playing an important rôle in preventing the passage of water to the higher parts of the plant. High absorptive power of this slime would explain how the organism may enter comparatively firm vessels. Somewhat below the epidermal layer of the pea stem there occur normally four cavities which permit a very rapid and abundant development of bacteria and bacterial slime. The high pressure

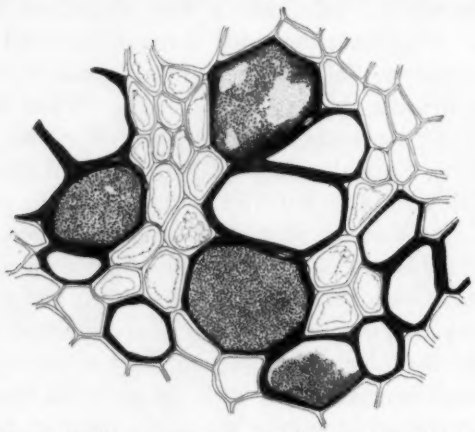


FIG. 6.—Cross-section of vessels occupied by bacteria (×1,000).

produced by the slime tears the surrounding tissue, producing large cavities (Fig. 4). In the surrounding parenchyma the organism can be found both intercellular and intracellular, enlarging the occupied area in the manner mentioned. In longitudinal sections of the stem the same process can be seen near the vascular bundles, and the pushing activity of slime and bacteria is visible in lens-shaped cavities which contain ruptured vessels either in or at their margin. However, this does not seem to be the only method of penetration and spreading of the organism in the plant, because in some instances it was possible to find that the bacteria dissolved the wall between two vessels, and penetrated from one into the other. The large cavities produced by the organism in the parenchyma of the pith (Fig. 5) and cortex can not be entirely accounted for by rupturing and crushing of tissue. Some parts of the cavity at least appear to result from solvent action of the organism.

Although under field conditions the organism is largely a parenchyma invader, it sometimes may enter the vessels. It is of special interest to

point out that where the stipules and leaves show large lesions at their bases, the vessels of the leaves and leaf traces and sometimes the vascular bundles of the stem are occupied by bacteria. This was found to be true not only in greenhouse experiments but also in the material collected in the field.

As has been stated by Sackett and later confirmed by Ludwig, the organism very readily enters the plant through wounds. Sackett has shown that stomatal penetration into the leaves may also take place. Experiments performed during the present investigations have confirmed the statements of the above-mentioned workers. We have succeeded in securing abundant stomatal infection on leaves (Fig. 8), but only in very few instances does it appear that stomatal penetration has taken place on the stem and pods.

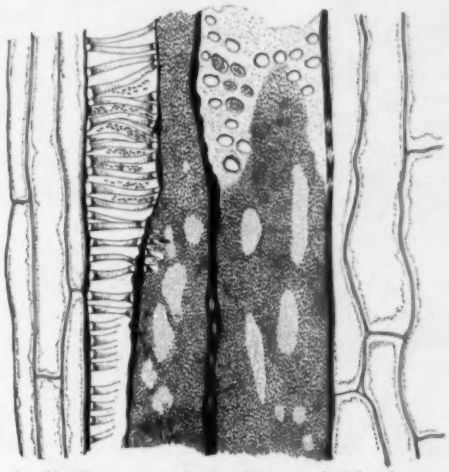


FIG. 7.—Longitudinal section of vessels occupied by bacteria (×1,000).

Even when the plants were kept for 48 hours in a moist chamber before inoculation to favor the penetration of the organism through stomata into pods and stems, negative results were obtained. It is worth mentioning that the staining reactions of the guard-cells in pods show differences in comparison with those in leaves, but it is not possible at present to determine their nature.

HOST RANGE

Sackett has shown that the organism attacks field and garden peas, and that it does not attack alfalfa, sweet clover, crimson clover, mammoth clover, cow peas, and garden beans. We have inoculated many other species in addition to those tried by Sackett, as shown in table 4. These pathogenicity tests have partly confirmed earlier findings by Sackett, but they have shown

at the same time that there are other leguminous plants susceptible to the organism. *Lathyrus odoratus* and *L. latifolius* showed symptoms quite similar to those in garden and field peas, with the difference that, although abundant stomatal infection was produced, the lesions were comparatively small. *Dolichos lablab* showed slight stomatal invasion and long black streaks along the stems and petioles. These streaks under microscopic examination have shown that the organism occurs principally in some of the vascular bundles, the surrounding parenchyma being invaded only to a small extent. The plant reacted to this attack by plugging of the vessels

TABLE 4.—*The results of inoculating various leguminous plants with Pseudomonas pisi*

Plant tested	No. plants inoculated	No. of checks	Result of inoculation[a]	Result of reisolation and reinoculation into pea plant[a]
Pueraria thunbergiana	3	1	–	
Phaseolus aconitifolius	4	1	–	
P. angularis	2	1	–	
P. vulgaris	6	2	–	
Melilotus officinalis	5	1	–	
Medicago sativa	8	5	–	
M. lupulina	6	3	–	
Trifolium incarnatum	2	1	–	
T. pratense	2	1	–	
Cicer arietinum	3	1	–	
Lens esculenta	4	1	–	
Lespedeza bicolor	5	1	–	
Arachys hypogaea	5	3	–	
Lupinus luteus	2	1	–	
L. albus	3	1	–	
Vicia faba	5	1	–	
V. gigantea	15	6	–	
V. villosa	12	5	–	
V. sativa	10	2	–	
V. pannonica	14	4	–	
Dolichos lablab	9	3	+	+
Lathyrus odoratus	14	6	+	+
L. latifolius	6	2	+	+
Lathyrus, wild sp. from Utah	5	2	–	
Progressus, white cow pea	4	1	+	+
Early black cow pea	5	1	+	+
New Erg. cow pea	5	1	+	+
Holly Brook soy bean	3	1	–	
Wisconsin black soy bean	3	1	–	
Pisum sativum	14	5	+	+

[a] + = Infection. – = No infection.

and abundant cell divisions around the invaded vascular bundles. The disease developed on cow peas in a similar manner as in *Dolichos lablab* except that there was no stomatal penetration. The fact that *D. lablab* is a host plant for bacterial blight of bean (*Pseudomonas phaseoli*) suggested that it might be possible to infect beans with *Pseudomonas pisi* reisolated from this host. Repeated trials to inoculate beans with strains of *P. pisi* obtained from *D. lablab* have failed to produce the disease.

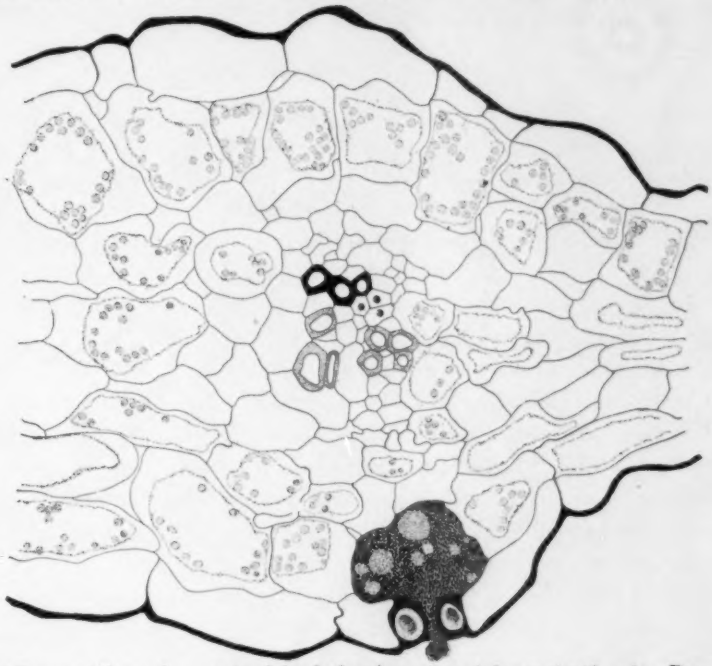


FIG. 8.—Cross-section of a part of leaf showing stomatal penetration by *Pseudomonas pisi* (×1,000).

The cultures of the organism used in inoculating other leguminous plants were at the same time used in inoculating peas and in every case produced the disease. In all cases where the disease appeared on inoculated leguminous plants, the organism was reisolated and produced infection on inoculated pea plants. Furthermore, on culture media, the organism reisolated from these plants has shown its identity with bacterial blight of pea.

CONTROL

The proof that pea blight is seed borne, and evidences of its transmission in this way to young plants both in the greenhouse and under field conditions, should aid in elaborating control measures. More attention should

be given to the pea fields from which the seed is to be selected for the subsequent year. The fact that the organism overwinters on the surface of the seed and within the seed coat would appear to necessitate disinfection of seed with substances which have a lasting bactericidal value. Such substances would kill not only the bacteria on the surface of the seed, but also would prevent the infection of the young seedlings by the organism which from time to time diffuses from the seed coat. Extensive tests with different disinfecting agents should be made.

SUMMARY

Infection by *Pseudomonas pisi*, the causal organism of bacterial blight of pea, often takes place on sepals, spreading towards the peduncle and pods, killing flowers, and causing shrivelling of young pods. Badly infected pods contain seed which often have more or less extensive bacterial films on the surfaces, and sometimes show a water-soaked spot near the hilum.

During moist weather, bacterial ooze is present. The bacteria exude from the infected plant at first in the form of short cirrhi, which later absorb water, forming droplets of slime containing bacteria.

The organism overwinters in the form of a dry bacterial film on the surface of the seed and also in the seed coat. It remains alive at least for ten months. When infected seed are sown, a certain percentage of plants coming from them will be infected, and such infected plants are centers for the dissemination of the disease in the field.

The organism penetrates through wounds, but stomatal infection on leaves and sepals occurs abundantly. Spreading through intercellular spaces, it enters readily into the parenchyma cells not only of the cortex, but also of the pith. In case of favorable conditions for the disease, the bacteria produce large cavities in the plant, breaking down cell walls by high pressure of bacterial slime and by chemical action. The important effect of this action is the breaking of vessels and entrance of the organism into the vascular bundles, with consequent wilting of leaflets and occasionally of the whole plant.

The entrance of the bacteria into the pod seems to be largely through wounds. Spreading between and through the cells, they penetrate through the pod wall and form abundant bacterial slime on the inner side of the pod and on the surface of the seed. The migration of the organism into the seed comes about only by passage from invaded pod tissue into the funiculus, and from there through the micropyle into the seed coat, where it remains alive in a dormant condition.

The organism is pathogenic not only to field and garden peas but to hyacinth bean (*Dolichos lablab*), cow peas (*Vigna sp.*), sweet pea (*Lathyrus*

odoratus), everlasting pea (*Lathyrus latifolius*), and probably to some other species of *Lathyrus*.

Seed treatment by suitable disinfectants would seem to offer possibilities for the control of the disease.

FACULTY OF AGRICULTURE AND FORESTRY,
UNIVERSITY OF ZAGREB,
ZAGREB, JUGOSLAVIA.

LITERATURE CITED

1. CARSNER, E. Angular-leafspot of cucumber: dissemination, overwintering and control. Jour. Agr. Res. **15**: 201. 1918.
2. JENNISON, H. A. Observations upon the bacterial blight of field and garden pea in Montana. Phytopath. **11**: 104. 1921.
3. JONES, F. R., and M. B. LINFORD. Pea disease survey in Wisconsin. Wis. Agr. Exp. Sta. Res. Bul. 64. 1925.
4. LUDWIG, C. A. *Pseudomonas* (*Phytomonas*) *pisi* Sackett, the cause of a pod spot of garden peas. Phytopath. **16**: 177. 1926.
5. SACKETT, W. G. A bacterial stem blight of field and garden peas. Col. Agr. Exp. Sta. Bul. 218. 1916.

EXPLANATION OF PLATES

PLATE XXI

A. Ripe pea seeds with water-soaked lesions near the hila.
B. Symptoms of bacterial blight on pea plant grown from infected seed.

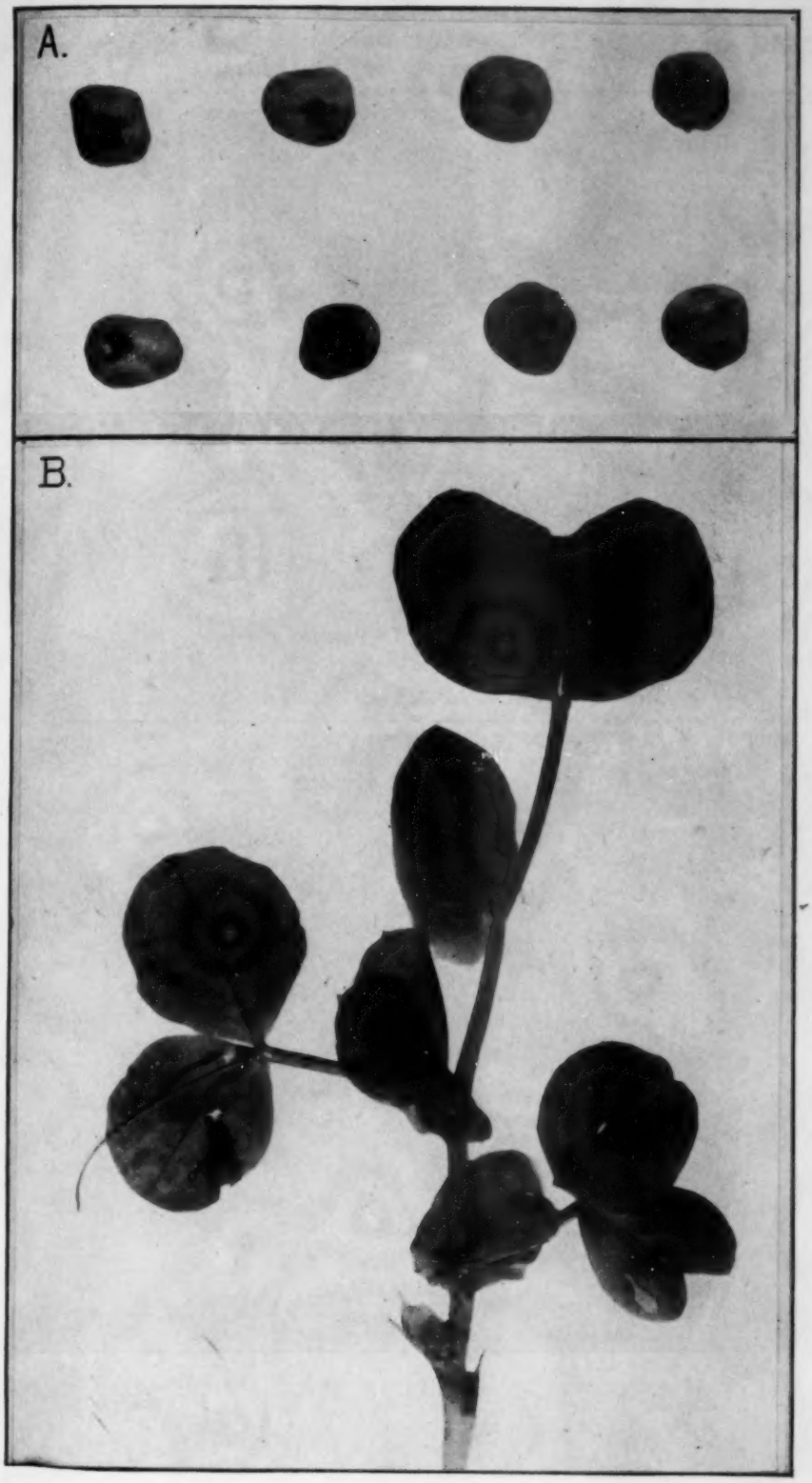
PLATE XXII

A. Pea plant in the foreground showing bacterial cirrhi on the stem.
B. Pea plants showing bacterial ooze.

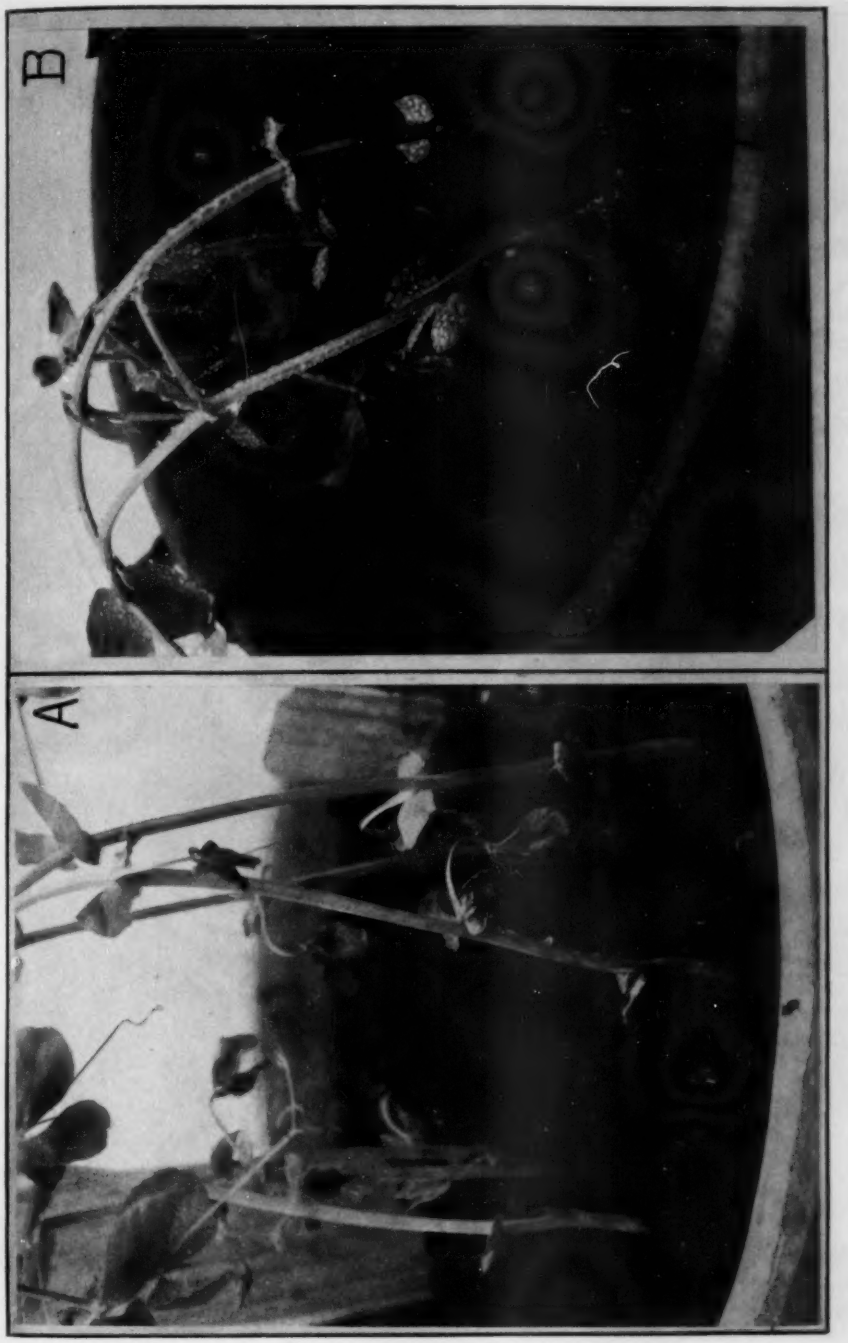
PLATE XXIII

Pea plant showing the infection on pods and the peduncle.

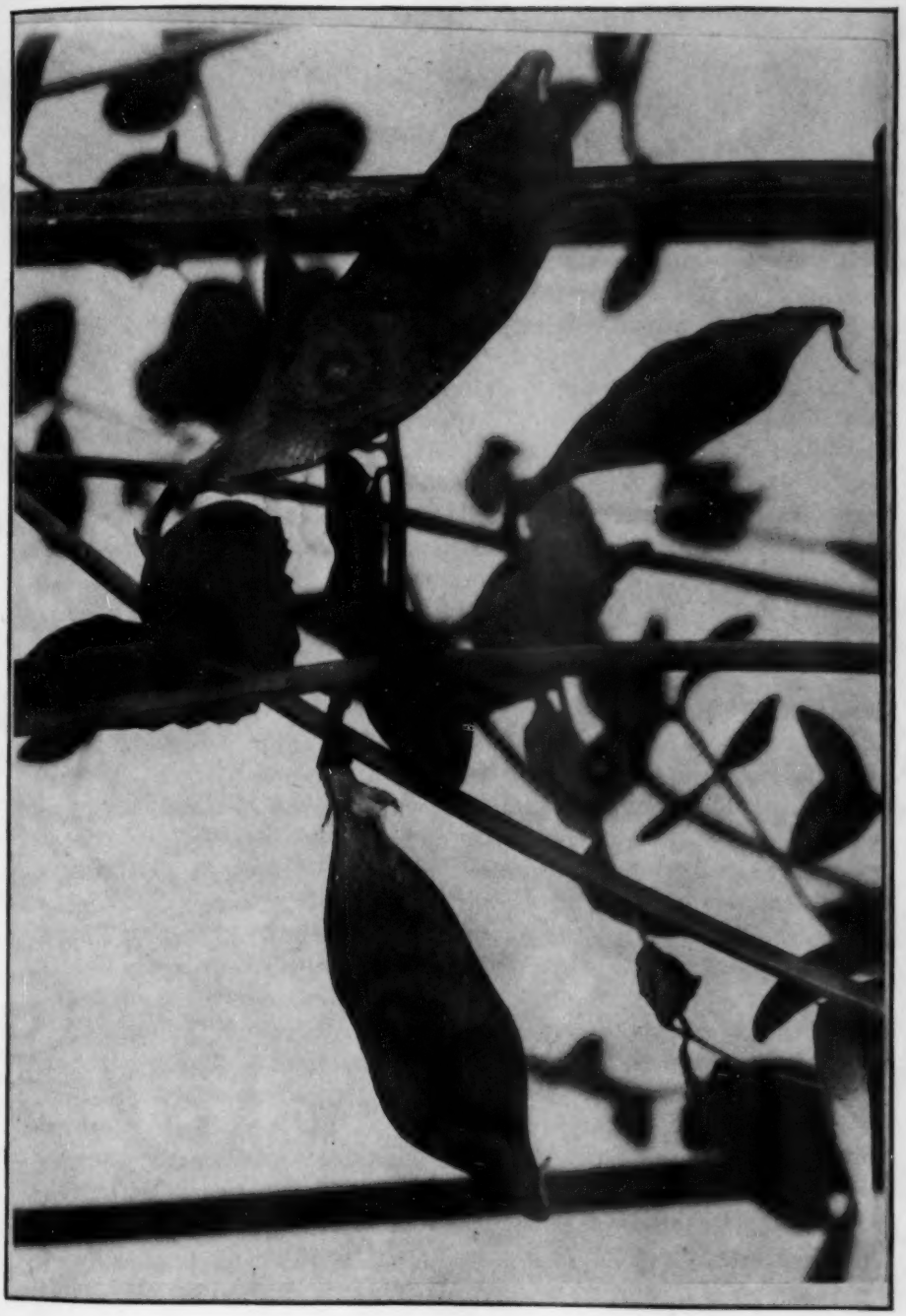
UNIV. OF MICH.


A.
B.

UNIV. OF WASH.


B
A

UNIV. OF MICH.



UNIV. OF MICH.

DWARF OF BLACKBERRIES[1]

S. M. Zeller

INTRODUCTION

A dwarfing disease of the vining type of blackberries represented by the Logan and Phenomenal varieties has been known to exist in the Pacific Coast states of California, Oregon, and Washington since 1918. In that year Darrow (2) mentioned in a discussion of the Phenomenal blackberry that "in many sections, because of a disease which results in a dwarfed growth of the plants, only two or three crops can be harvested before the plantation becomes unprofitable. . . . Compared with the loganberry the plants are more subject to the dwarfing disease and are shorter lived." Although dwarf has been known on the Coast for some years, it is only recently that the disease has received detailed attention by the writer. His observations of the symptoms and nature of the disease and methods for its control are recorded in this paper.

DISTRIBUTION AND OCCURRENCE

The geographic distribution of the dwarf disease is limited so far as known to various localities in California where Logan and Phenomenal berries are grown, to the coastal slope, Willamette and Umpqua valleys in Oregon, and the portion of Washington west of the Cascade Mountains. The disease has been reported from practically every region of these coastal states in which Phenomenal blackberries are grown. In fact the occurrence of the disease seems to be associated definitely with the Phenomenal berry but usually spreads to the Loganberry when the two varieties are planted in close proximity. The accompanying map of Oregon (Fig. 1) shows the stations where dwarf has been observed or from which specimens have been sent to the writer for identification.

The studies on dwarf by the writer have been conducted in the main in Oregon, and so the consideration of the occurrence here given is based principally on Oregon conditions. In some plantings affected by the disease the damage is slight or relatively unimportant, while in others the losses of plants vary from serious to total. The losses have been so serious in some Loganberry plantings that the entire acreages have been grubbed out, while 100 per cent of the plants were found diseased in one planting containing

[1] Published by permission of the Director of the Oregon Agricultural Experiment Station.

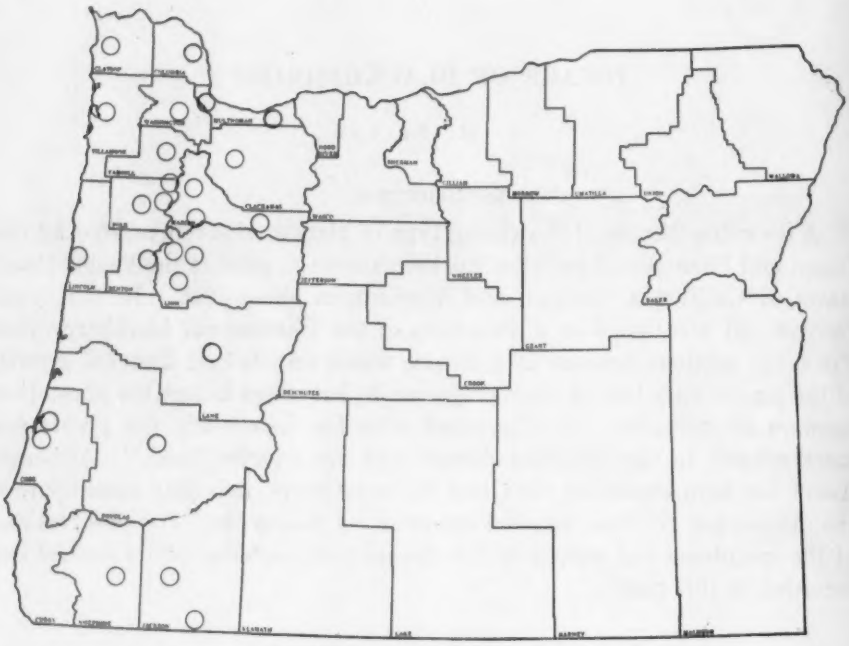


FIG. 1.—Map of Oregon showing the stations at which blackberry dwarf has been observed or authentically reported to the writer.

600 Phenomenal plants. In one case near Corvallis there were several diseased plants in one row of Phenomenals along one side of a three-acre planting of Loganberries. The disease gradually spread into the Loganberries until all of the Phenomenals and about 50 per cent of the rows of Loganberries adjacent to them have been removed, but still there are scattering plants in the remaining rows which manifest symptoms of dwarf. At any one time during the last four seasons the highest count of diseased plants in this case was 6 per cent. In a three-year old planting of Loganberries in Lane County 19 per cent of diseased plants were found. The owner stated that the diseased plants had increased in number for three years. The tips for the planting had been purchased from a grower who had raised both Logan and Phenomenal berries. Many other similar cases might be cited, and many cases of very low percentages of diseased plants have been found. Few plantings of the Phenomenal blackberry have been found without dwarf, but Loganberries planted alone seldom have the disease. Dwarf has not been found in Loganberries except in cases where its origin could be traced directly to the Phenomenal or to its probable transmission from it.

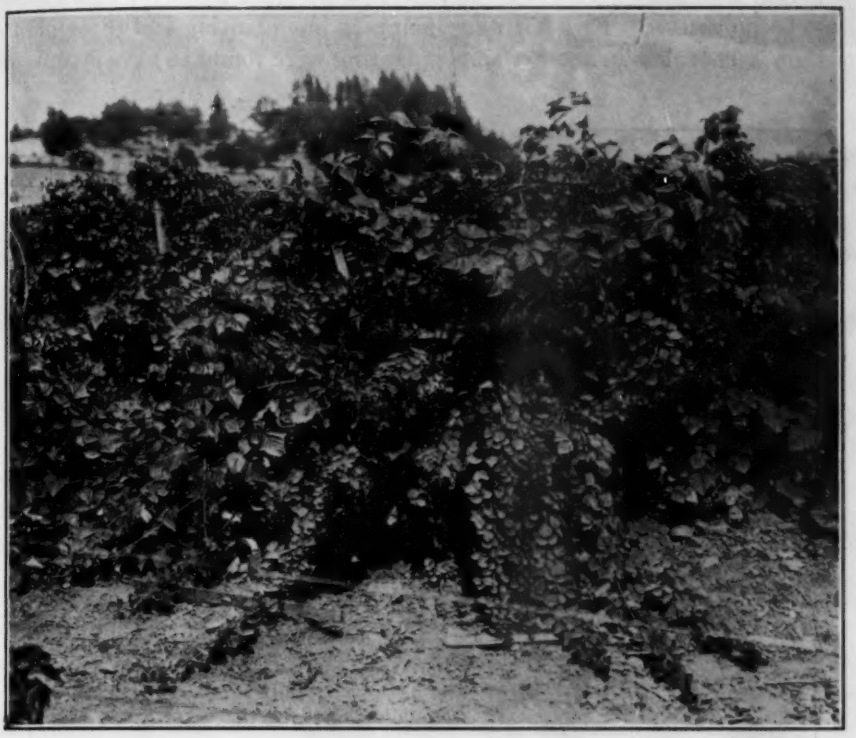


FIG. 2. The first noticeable symptoms of dwarf in a Loganberry plant infected after it was mature. The canes are spindly with small leaves which usually have a normal color. The healthy cane is thrown over the top for the sake of comparison.

ECONOMIC IMPORTANCE OF DWARF

The economic importance of the dwarf disease is measured essentially by the percentage of plants affected. A plant which has had dwarf for one full season is valueless. Therefore the economic losses are really shown in the examples mentioned above. Taken as a whole, the loss through dwarf to the Loganberry industry in the three Pacific Coast states is very slight, but many individual growers have been found to have a high enough percentage of infected plants to make the remainder of healthy plants fall far below a financially successful planting, making total eradication necessary.

HOST PLANTS

The Phenomenal and Loganberry are both susceptible to dwarf, the disease being practically limited to these two berries. The Phenomenal is by far the more susceptible. With the exception of a few plants each of the

Kittitany and Cory's Thornless blackberries, no other hosts for this disease have been observed. Five Kittitany plants in one planting and three plants of Cory's Thornless in another single planting were found to have symptoms

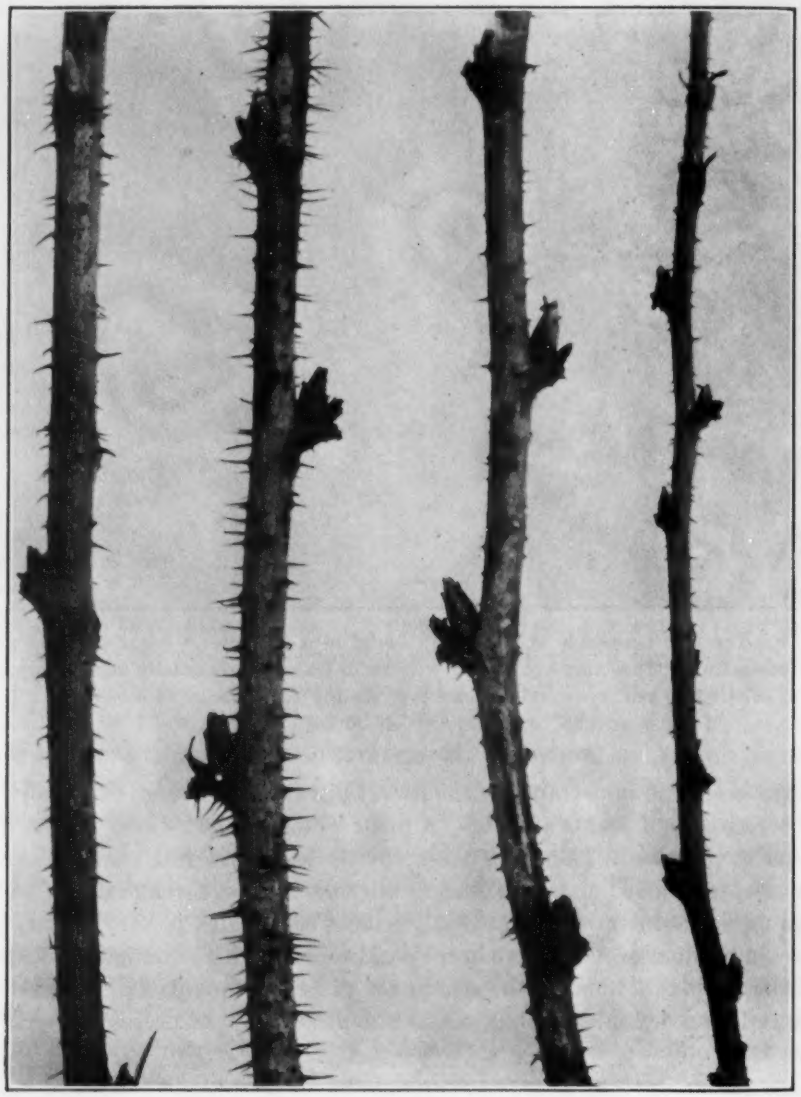


FIG. 3. Portion of a healthy Loganberry cane (left) and portions of three canes (right) from dwarfed plants. Notice the shortened internodes of the diseased canes. There are usually more than one bud at a node on the diseased canes and one bud at a node on the healthy canes.

similar to dwarf, and, although transmission experiments have not definitely proved the disease in these two varieties to be dwarf, the symptoms and behavior of the plants are the same.

SYMPTOMS OF DWARF

General Symptoms.—The progress of the dwarf disease in the plant is characterized by distinct morphological and physiological changes, most marked in the leaves and stems, and to a certain extent in the fruit. These consist principally of an abnormal color accompanied by certain malformations and a general dwarfing. The symptoms vary somewhat according to the age of the plants at the time of infection, but the general characters are the same in all stages of growth after the initial year of infection.

FIG. 4. Portion of a severely dwarfed cane of the Phenomenal blackberry taken just after the leaves had started in the spring. Notice the extremely short internodes and the great number of buds at some nodes.

Symptoms on Mature Plants Infected in the Field.—The first apparent manifestation of the disease in a mature plant is not so much a dwarfing of the stems as of the leaves. Instead of a foreshortening of the stems as may be apparent in subsequent years, the canes during the first year of noticeable symptoms are spindling and flexuous. The leaves are much smaller than leaves of healthy plants, and the leaflets are of abnormal shape—obovate instead of ovate as in normal leaves. At this stage the leaves are of a normal color and the whole plant presents an appearance as illustrated in figure 2.

In the fall of this first year there are several small buds, usually three or more, set at each node instead of the usual single bud in healthy canes. In the winter condition this characteristic of the buds presents quite an apparent contrast to those of healthy canes (Fig. 3). The next spring all of the buds at each node grow (Fig. 4) and produce short laterals, but one finally becomes predominant and the others dry up when they are about one or two inches long. The laterals produce flowering buds rather normally and a meager amount of fruit. After the first leaves are out this second

FIG. 5. Current season symptoms on a Loganberry plant which was infected the first year from the young tip. Notice the peculiar crinkling of the leaves. This plant was caged May 11, 1925, viruliferous aphids were introduced May 14, and on May 20 there was first noticed slight necrosis along the mesophyll between the lateral veins. First dwarf symptoms were noticed on June 6. Photographed July 17.

spring the canes take on a rosetted appearance due to the crowding of the leaves at the nodes. The new canes which are produced the second season are typical of dwarf plants in all of their later history (Figs. 10 and 11).

Symptoms on Plants Infected in the Young Tips.—The first symptom in all cases is necrosis, which appears in the young leaves upon which the viruliferous insects have fed. This necrosis may be slight or severe, as described later in this paper. Leaves which appear on all parts of the young plants after inception of the disease have a peculiar distortion and crinkliness

FIG. 6. This young Loganberry tip was caged May 11, 1925, and non-viruliferous aphids were introduced on May 14. This plant has remained healthy.

(Figs. 5 and 7) and are much smaller than on normal plants of the same age (Figs. 6 and 12). These leaf symptoms characterize a majority of the leaves produced during the remainder of the first season. The canes grow spindly and flexuous. The second season the plants (Fig. 8) take on the appearance of diseased mature plants in their first season, as described above, becoming typical dwarf plants the third season. The detailed symptoms following infection by insects will be discussed later in this paper in connection with transmission experiments.

Leaf Symptoms.—The pattern of coloring, shapes and distortions which the leaves of dwarf plants assume vary with the conditions under which the leaves are produced, or during the current year of infection the leaf symptoms vary with the age of the plant at the time of infection. The stage of development of individual leaves of course influences the appearance of the

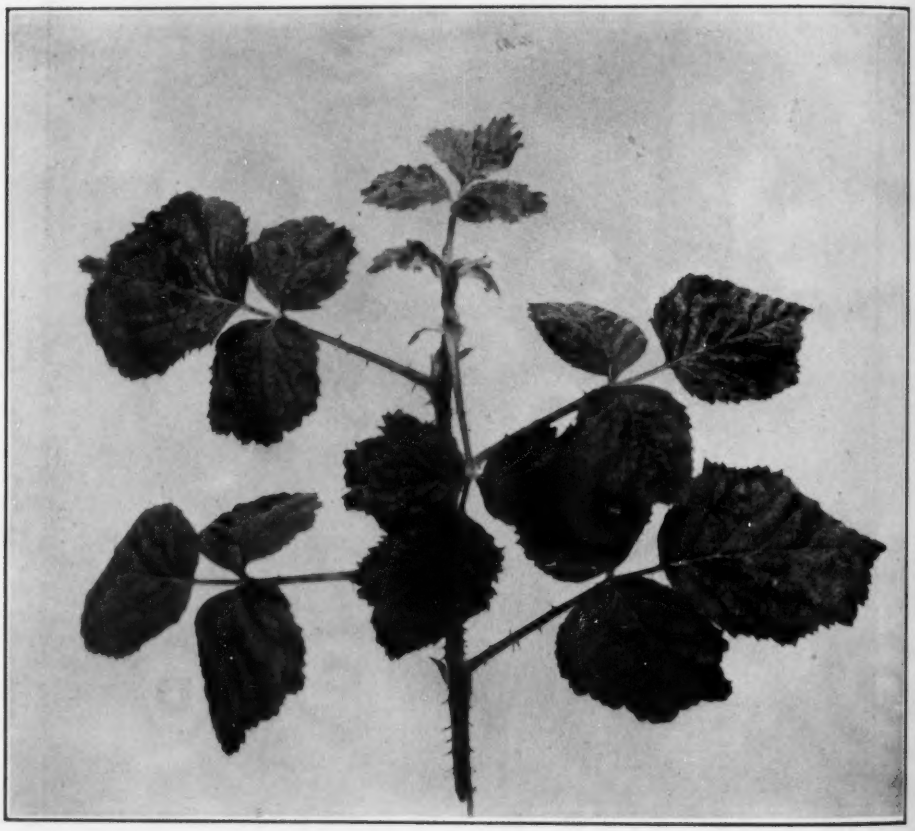


FIG. 7. An individual tip from the plant shown in figure 5, illustrating the mottled and crinkled or savoyed condition of the leaflets.

symptoms, for leaves produced during succulent growth are different in texture than those produced during more xerophytic growth.

Just after disease inception in young plants which are in their first year from tips, the leaf symptoms are as shown in figure 7. Previous to this condition there is necrosis, which will be described later in this paper. Following the necrosis all growth of the current season produces the crinkled leaves. It appears from the crinkling that the veins of the leaves grow less rapidly than the mesophyll, so that a more or less bullated condition follows the spaces between the primary veins. In many of such leaves the margins are extremely irregular. The leaflets become more orbicular and broadly ovate as the season progresses and even become obovate, in contrast to the graceful ovate leaflets of healthy plants. The leaves are not usually abnormal in color during the current season of infection.

When older healthy plants are infected, the leaves are not so crinkled as just described. They are much reduced in size and very regular in margin and surface characters, but are not much lighter in color than the healthy leaves.

On the contrary the leaves in the more advanced stages of dwarf have more characteristic symptoms. These symptoms are influenced by climatic factors. The leaf symptoms of succulent growth such as produced under greenhouse conditions or during the vigorous growth of the damp spring months are distinct. Leaves grown under such conditions are larger than those leaves of diseased plants grown later in the summer. They are not deeply crinkled but are wrinkled or bullated in smooth undulations, owing perhaps to the more or less marbled occurrence of the mottling. This marbled mottling is made up of irregular splotching of bronzed-green mesophyll and lighter (chlorotic?) areas. The succulent leaves grown in half shade, as in insect-proof cages, are very finely mottled. Figure 9 shows this mottling, which is the predominating type of chlorosis found in the leaves of dwarfed plants. The contrast with the clear green of the healthy leaf is striking. The leaves shown in figure 9 were grown under cages and were photographed in July. The finely-netted mottling follows rather regularly the netting of the finer veins of the leaf. Tips and margins of the leaflets are usually darker green than their central portions. The margins are not so uniform in outline as those of the healthy leaflets. The mottling of diseased leaves produced in the open during warm dry weather is not very different from that just described, but the leaves are much more rigid—the margins and apices of the leaflets have more of an upright tendency, producing a cupping of the leaflets (Fig. 11). The margins of such leaves are stiff and much less desirable to handle than healthy leaves.

Stem Symptoms.—The stems of dwarfed plants are not mottled or streaked in any way, nor is there any type of necrosis of the bark or inner

tissues. The color of the diseased stems appears as that of normal stems. The spindliness of the canes and shortening of the internodes are the chief characteristics of canes in the early stages of the disease (Fig. 3). Later the canes are extremely foreshortened, characteristically stout and stiff, with extremely short internodes (Figs. 4, 10 and 11). The new canes come later in the spring than those of healthy plants and are fewer in number each year. There are often several buds at each node.

Many canes of dwarf plants have been layered in the fall but they do not readily take root. The spindly canes of the first year may root, but such rooted tips are very weak and seldom grow any sprout.

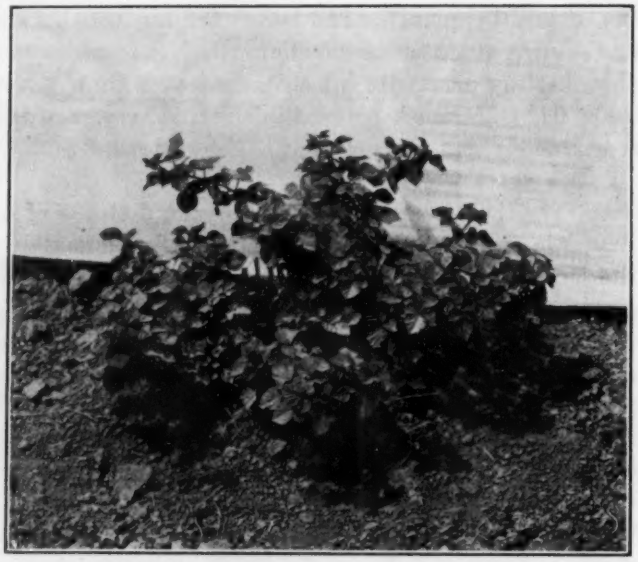


FIG. 8. The same plant as that illustrated in figure 5, showing the characters of dwarf one year later. Notice the small rounded leaflets and the snubbed tips of the canes. Photographed July 21, 1926.

Fruit Symptoms.—In the less extreme cases of dwarf, fruiting laterals may grow to nearly normal length and set nearly as much fruit as do healthy laterals. The only appreciable abnormality in the flowers is the reduced size of the sepals and petals. The druplets of the fruit sometimes ripen a little unevenly. The fruit develops to a fair size, but there is a tendency for the druplets to fall from the receptacle. Some growers say the berries fall apart and become a crumbly mass in the boxes.

Root Symptoms.—The roots of dwarfed plants show no external or internal evidence of the disease except that in its later stages there is a reduction

in the number of the larger roots and finer rootlets. The tips of canes which are layered do not readily take root. The extremely inferior plants so produced do not persist for long.

NATURE OF THE DISEASE

The symptoms and behavior of the dwarf disease of blackberries indicate that it is one of the infectious chlorotic diseases. Sufficient data have been gathered upon which to base a positive statement as to the infectious nature

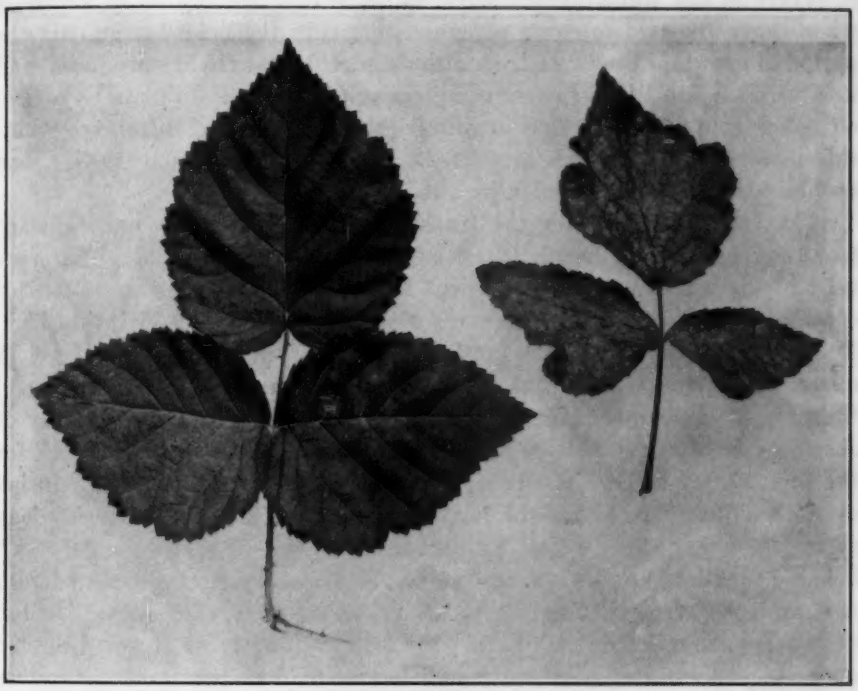


FIG. 9. Differences in the size, margins and markings of the leaflets of healthy (left) and dwarfed (right) leaves of the Loganberry. Notice the finely-netted mottling of the diseased leaf. Photographed on panchromatic plate, using Wratten A filter.

of the disease. No fungous or bacterial organism has been found associated with the dwarf disease, nor have any foreign cell inclusions been found as yet. Transmission of the dwarf disease, however, has been accomplished by means of aphis (8).

TRANSMISSION OF THE DWARF DISEASE

The general appearance of diseased plants and the gradual spread of the disease year after year led the writer to believe it to be transmitted by some

agent in the field. This supposition led to the first inoculation experiments in 1925. These were repeated in 1926.

Early in April, 1925, twenty-one young tip plants of Loganberry were covered with cages. Vigorous cane growth had already begun. The cages eliminated the possibility of outside infection, particularly from insects, during the spring and summer period of active growth; and the shading aids succulency in the plants, a condition favorable to infection by means of sucking insects.

There is one unfortunate factor which enters inoculation experiments when cages are used to cover perennial plants in the field during only the period of growth. Under such conditions, succulent canes are produced, and it is necessary to lift the cages early enough to allow the plants to harden off before cold weather. This introduces the possibility of infection by outside insects after the cages are lifted. In my experiments there has been no indication of such infection by outside agencies.

The location chosen for the transmission experiments is quite distant from any planting of horticultural varieties of bramble fruits. The cages used in the work are of a convenient size to cover one plant and allow of considerable growth. The cages are 20 inches square and 36 inches high. The lumber for the frames is finished so that it will not catch in the cloth during the process of covering. There is a base board of 1×8 material, and the uprights and upper rim are of 1×3 material. A very good quality of sheeting (LL Caddo) is used to cover the frame. A piece of the cloth for the top, 36×44 inches, has its margins tacked so as to lap about 2 inches over the sides. This allows the top to bag so that a slit about 6 inches long across its center may be tied with a string. This slit serves as a "peek" hole for making observations and doing the necessary work connected with inoculations. A piece of cloth 88×36 inches serves for the sides. The selvage edges are rolled enough to take up the slack and tacked around the top and around the upper part of the base board. The ends are rolled together and tacked along one of the uprights. Then all of the tacked edges are nailed down with lath so that all are insect-proof. The cages are set in place over the plant and the base board is covered with soil up to the lath holding the lower edge of the cloth. Except for size and shape this is the type of insect cage used for caging potato plants by Schultz and Folsom (5) and by McKay of the Oregon Experiment Station.

It is still an open question as to how the dwarf disease is transmitted from plant to plant under natural conditions. The aphis, *Aphis rubiphila* Patch, which is reported by Rankin (4), and *Amphorophora rubi* Kaltenback, reported by Wilcox and Smith (7), Berkeley and Jackson (1), and by others, as carriers of the virus diseases of *Rubus* in the Eastern and Middle Western States and Eastern Canada, have not been found west of

the Cascade Mountains in Oregon or Washington. These species of aphis may be in this section but surely they are extremely scarce. Only one collection of each of these species has been reported as taken in Pacific Coast States. Both of these were in San José, California. It is not improbable that the long dry summer seasons, without the intermittent rainfall which is so common in the regions where these species abound farther east, is a factor limiting their propagation here.[2]

Thus we were at a loss to know what insects to use as a transmitting agency in our experimental work with blackberry dwarf. As aphis, leaf hopper, and such sucking insects are usually the carriers of virus diseases,

FIG. 10. A Loganberry plant in about the third year of dwarf. The longest canes here are about 20 inches long. Notice the rosetted or snubbed ends of the canes. This plant is seven years old.

[2] During the spring months of 1927, after this paper had been completed, the author found *Amphorophora rubi* on the Himalaya blackberry near Corvallis and Canby, Oregon.

the writer finally decided to make transmission trials with insects usually found on *Rubus* or some other Rosaceous plants. In 1925 aphids were used; in 1926, aphids, leaf hoppers, and tree crickets were given a trial as agencies for dwarf transmission.

FIG. 11. A Loganberry plant in the fourth year of dwarf. The canes were 10 to 12 inches long.

Transmission Trials with Aphis

It was thought most probable that aphids infesting some other Rosaceous host would most readily pass to species of *Rubus*. It was found that the wild sweetbriar rose (*Rosa rubiginosa*) so commonly found in the Willamette Valley was usually infested with aphis. On April 1, 1925, a colony of these was transferred to a cage containing a healthy Loganberry plant. This proved later to be a mixed colony of *Capitophorus tetrarhodus* and *Macrosiphum dirhodum*. This mixed colony was used as the dwarf-transmitting agency in 1925. In about two weeks (April 15) some of the aphids were transferred to a cage containing a Loganberry plant which was extremely dwarfed. This dwarfed plant had been obtained from Gresham, Oregon, early in April.

The 21 healthy Loganberry tips which had been planted out and caged early in April were taken from a disease-free planting which had been under the writer's supervision since the spring of 1924. The original stock was taken early in the spring of 1924 from a Loganberry planting which was

extremely isolated in a valley in the eastern part of Douglas County, Oregon. This whole planting was extremely vigorous and free of any symptoms of virus diseases.

To ten of the cages containing healthy Loganberry plants were transferred aphids from the caged dwarf plant, and to six of the cages aphids from the caged healthy plant were transferred on May 14. From four to ten individuals were transferred in each case. In order to secure the aphids from one plant and place them on another, the leaves of the first plant upon which they were feeding were picked and laid with care on a very young, succulent leaf of the second plant. The next day observations were made and it was found in all cases that the aphids had crawled from the picked, wilted leaf to the leaf of the healthy plant. Observations were made each day for several days in order to detect the first symptoms of disease. On May 20 the first effect of the viruliferous aphids was to be seen. On the leaves of some of the plants there was very slight necrosis along the veins and particularly in the mesophyll between the lateral veins where the aphids were feeding. As the necrotic areas increased, the aphids moved on to more succulent tissues. In a few cases the necrosis was extreme, while in others it involved only the leaf on which the viruliferous aphids were feeding and all of the newer leaves and younger part of the cane above. In one extreme case a young plant was killed to the ground and not until July 17 was there any sign of life. On that day there was observed a tiny new shoot coming from below ground. In late August this had developed into a very dwarfed, chlorotic cane about three inches long. The cage was gradually removed so as to allow the plant time to become hardened to the sunlight. The plant lived until late in December, but died before the spring of 1926, although the winter was extremely mild.

Seven of the ten plants receiving colonies of aphids from the diseased plant showed symptoms of dwarf before the summer months passed. None of the six plants receiving aphids from the healthy Loganberry plant showed symptoms of dwarf and grew normally in the spring of 1926.

After necrosis had been observed there were no abnormalities appearing until subsequent new growth appeared. On June 8 the first dwarf symptoms were noticed in one plant, and soon after that in five other plants. The new leaflets were slightly more rounded than normal, and some crinkling was appearing. By June 20, symptoms of first year dwarf were apparent. On July 17 the plant illustrated in figure 5 was photographed. The symptoms shown there were characteristic of six of the seven plants affected. At this time new canes were starting and the leaves on these were mottled and showed more typical dwarf symptoms. In all cases the canes upon the leaves of which the aphids had been placed did not elongate further. The short laterals they threw out were very ragged appearing, owing to the severe

crinkling and laciniation of the margins of the leaves. The newer canes were elongating considerably by July 28. The leaves were mottled in a splotchy manner by this time, and the whole appearance gave the impression that the entire plant would succumb.

The next year (1926) these plants appeared as shown in figure 8. One year after infection all the six remaining plants showed typical dwarf symptoms.

FIG. 12. Two tips were planted in this hill; one has been infected with dwarf and the other remained healthy.

In the spring of 1926 four caged Loganberry plants were inoculated by transferring to them viruliferous aphids from a dwarfed Loganberry plant. This was done as described above for the 1925 experiments. In this case a pure colony of *Capitophorus tetrarhodus* was used as the transmitting agency. Three of the four inoculations were successful and the progress of the disease in these cases the first year is comparable to that described above. Thus, from the experiments of the two years there have been ten out of fourteen plants to which the disease has been carried by viruliferous aphids, while ten plants to which non-viruliferous aphids from healthy Loganberry plants had been transferred remained healthy.

Transmission Trials with Tree Crickets

The snowy tree cricket (*Oecanthus niveus* De Geer), Race "B," as described by Fulton (3), is so common in plantings of blackberry in Oregon that it was thought to be a possible carrier of the dwarf disease.

Loganberry canes with many egg punctures were collected about May 15, 1926, and some were placed in a cage with a healthy Loganberry plant and others in a cage covering a dwarfed Loganberry plant. The eggs hatched and nymphs matured in these cages. The adult females began ovi-

positing late in July. On August 4, 1926, females which were actively ovipositing were taken after they had deposited several eggs. Two or three of these were transferred to each cage covering healthy Loganberry plants. Four cages received tree crickets which had been ovipositing and feeding on plants affected with the dwarf disease, and four cages received females which had been ovipositing and feeding on healthy Loganberry plants. When these eight plants were examined again in the early part of September, they had all been punctured by the tree crickets, but in no case were there any abnormal symptoms. All the plants appeared healthy late in November. As a result of this experiment the writer does not believe that the dwarf disease is disseminated by the snowy tree cricket.

Transmission Trials with Leaf Hoppers

Leaf hoppers were also tested as possible carriers of the dwarf disease. A large number of a white leaf hopper, probably the rose leaf hopper (*Empoa rosae* L.), were caged on a healthy and on a dwarfed Loganberry plant on July 27, 1926. On August 4 a number of hoppers were transferred to each of eight cages of healthy Loganberry plants, four receiving hoppers from the dwarfed plant and four from the healthy plant. None of these plants had shown dwarf or any abnormal symptoms by November.

Transmission Trials by Other Methods

In 1925 and 1926 a total of eleven healthy Loganberry plants have been inoculated in several different ways with the juice from succulently growing dwarf plants. To obtain this juice the leaves were macerated and ground in a mortar with just enough sterile distilled water to moisten the tissue very slightly. The juice thus obtained was drained off into a bottle and used in the following four ways: (a) Juice was placed on leaves near the growing tips of canes, and punctures were made in the leaf with a needle so that the juice entered the tissues of the healthy leaf. (b) Drops of the juice were placed in the crotch of the leaf petioles next to the axillary bud and the juice was allowed to penetrate the tissues next to the bud through needle punctures. (c) Where canes were forked with two branches, one branch was cut off leaving a stub about 2 inches long. This stub was so placed in a vial containing some of the juice that the cut end could absorb the juice. (d) Juice was injected into the pith and woody cylinder of canes by means of a hypodermic needle.

In no case, as a result of these inoculations, have any abnormal symptoms developed.

In the summer of 1924, buds from dwarfed Logan and Phenomenal plants were budded into healthy Loganberry, Cuthbert red raspberry, and

Munger and Plum Farmer black raspberry. This budding was done by Mr. Lyle Wilcox of the Department of Horticulture, Oregon Agricultural College. None of these buds lived over until the next year owing to winter kill of the canes, but many of them were apparently vigorous until late in the fall. There were no indications, however, of dwarf transmission.

INFLUENCE OF ENVIRONMENT ON DWARF

No particular experiments have been planned to ascertain the relation between climatic or edaphic factors and dwarf symptoms. From the practical standpoint of roguing it should be said that if a plant has ever suffered from dwarf it will retain enough of the symptoms even in winter condition so that the diseased plants may be recognized. The difference between hot weather symptoms and the succulent growth symptoms produced under more favorable moisture and temperature conditions have been discussed in another part of this paper. An erratic elongation of canes of dwarfed plants seldom occurs. Elongation has been noticed during short cool, humid periods, but whether in these cases it was a direct effect of the observed conditions has not been checked experimentally. Increased fertility, such as the addition of horse manure to the soil, has not materially changed the growth of dwarf plants.

COMPARISON WITH SOME OTHER VIRUS DISEASES OF BRAMBLES

As far as the writer is aware, the dwarf disease of blackberries is distinct from the other virus diseases of *Rubus*. It has some symptoms which are similar to some of those of the streak disease described by Wilcox (6) as Eastern blue stem, and the type of mosaic of black raspberry which has been designated as "yellow mosaic" by Dr. C. W. Bennett of the Michigan Agricultural College in an unpublished bulletin. The tips of the canes in dwarfed Logan or Phenomenal plants have the snubbiness which is characteristic of these two diseases in Middle Western States. Black raspberry plants having the red raspberry mosaic may exhibit a similar snubbiness of the tips of the canes, as do also black raspberry plants which become affected slowly with the wilt disease (*Verticillium*). The mottling of the leaves of dwarfed plants is not entirely different from that of yellow mosaic but is entirely different from that of streak.

CONTROL OF DWARF

Since the tips of canes of diseased plants do not readily take root, even when carefully layered, there is little danger of spread of the disease from planting to planting through nursery stock. The only spread in this way might be possible from the transplanting of rooted tips from apparently healthy plants which had been infected very late in the summer or autumn. It is believed, however, that if there were no other means of transmission than the rooting of tips the disease would soon be self-exterminating.

One of the most practical means of prevention of dwarf, as in the case of other virus diseases, is the planting of stock from fields which are free of the disease. Most Loganberry plantings in Oregon and Washington are free of dwarf, but where it exists in low percentages, less than 5 per cent for instance, it is practical and advisable to rogue out the affected plants. From three plantings of Loganberries the disease has been eliminated by two roguings in as many succeeding seasons. These plantings had approximately 1.5, 2.5 and 4.0 per cent infection, respectively.

All plantings of Loganberries where dwarf has been found have been near at least a few plants of the Phenomenals, or the stock from which the plants were taken had been in such a relation to the Phenomenal blackberry. The Phenomenal berry is used so little in a commercial way in Oregon that it should be eliminated from commercial plantings entirely. At least Loganberries should never be grown near Phenomenals.

SUMMARY

1. The dwarf disease of the vining type of blackberry (dewberry), represented especially by the Logan and Phenomenal varieties, was recognized in the Pacific Coast States as early as 1918.

2. The disease occurs wherever the Phenomenal berry is grown and also infects the Loganberry, Cory's Thornless and the Kittitany blackberry. It is most widespread where Loganberries and Phenomenal berries are grown commercially, *i.e.*, from British Columbia south to central California. A map of Oregon is presented to show the known distribution of the disease in this state.

3. The economic importance of the disease is measured by the number of plants affected, for a plant which has had dwarf for one full season is valueless thereafter. Some growers of Phenomenal berries have reported as many as 100 per cent of the plants affected by the third year in plantings which have not been rogued. One planting of Loganberries with 19 per cent of diseased plants in the third year has been found. As a rule, however, the loss through dwarf to the Loganberry industry in Pacific Coast States is very slight, but many individual growers have experienced high enough percentages to make total eradication necessary.

4. Dwarf is characterized in its severe stages by short, stubby canes, the internodes of which are very short. The whole cane has a very leafy appearance. The leaflets are smaller, more rounded, and lighter in color than normal, showing a finely-netted uniform mottling. The fruit develops to a fair size but the druplets easily fall apart, becoming a crumbly mass in the boxes.

5. Tips of canes do not readily take root when layered and it is believed the disease would soon be self-exterminating if there were no other means of transmission.

6. The dwarf disease is one of the infectious virus diseases as demonstrated by transmission experiments in which a mixed colony of aphis (*Capitophorus tetrarhodus* and *Macrosiphum dirhodum*) found on the wild sweetbriar rose, *Rosa rubiginosa*, were the agents of transmission. In another experiment the disease was transmitted by *Capitophorus tetrarhodus* alone. On the other hand, experiments with the snowy tree cricket (*Oecanthus niveus* De Geer) and leaf hoppers (probably *Empoa rosae*) resulted in no transmission. Juice expressed from macerated diseased leaves was injected into healthy plants in several ways with negative results. When buds from diseased plants were grafted into healthy plants, no disease resulted.

7. Environmental changes have had little influence on symptoms of dwarfed plants.

8. Dwarf of blackberries has some symptoms in common with such other virus diseases of brambles as the streak and yellow mosaic of black raspberries and the expression of red raspberry mosaic when infecting black raspberries. On the other hand, most of the symptoms of dwarf differ from those of other virus diseases of brambles.

9. Preventive measures are (a) to secure stock from plantings which are free of dwarf, (b) to discourage the use of the Phenomenal blackberry as a commercial or home garden variety in districts where the Loganberry is a desirable commercial product, (c) to rogue plantings with small percentages of dwarf, such as five per cent or less.

OREGON AGRICULTURAL EXPERIMENT STATION,
CORVALLIS, OREGON.

LITERATURE CITED

1. BERKELEY, G. H., and A. B. JACKSON. Studies in raspberry diseases; mosaic, leaf curl, rosette and wilt. Dominion of Canada, Dept. Agr., Pamphlet N. S. 72. 1926.
2. DARROW, GEO. M. Culture of the Logan blackberry. U. S. Dept. Agr. Farmers' Bul. 998. 1918.
3. FULTON, B. B. The tree crickets of Oregon. Ore. Agr. Exp. Sta. Bul. 223. 1926.
4. RANKIN, W. H. Report of the Dominion Field Laboratory of Plant Pathology, St. Catharines, Ont., Canada, Dept. Agr. Div. Bot. Interim Rept. 1921–1922: 30–60. 1922.
5. SCHULTZ, E. S., and DONALD FOLSOM. Infection and dissemination experiments with degeneration diseases of potatoes. Observations in 1923. Jour. Agr. Res. **30**: 493–528. 1925.
6. WILCOX, R. B. Eastern blue stem of the black raspberry. U. S. Dept. Agr. Circ. 227. 1922.
7. ———— and F. F. SMITH. Transfer of mosaic disease from red to black raspberries (Abst.). Phytopath. **14**: 55. 1924.
8. ZELLER, S. M. Preliminary report on transmission of dwarf of loganberry (Abst.). Phytopath. **15**: 732. 1925.

STORAGE ROTS OF CRANBERRIES IN THE 1926 CROP[1]

NEIL E. STEVENS AND HENRY F. BAIN

INTRODUCTION

The cranberry, *Vaccinium macrocarpon*, offers an unusual, perhaps a unique, opportunity for a nation-wide study of the relative importance of rot fungi under the various climatic conditions found in different parts of the United States. Commercial cranberry growing is concentrated in a few centers. The chief centers are, however, widely separated. Certain superior native varieties selected in the past have been used almost exclusively in planting the later bogs, with the result that the same varieties are found to some extent in all cranberry sections. Except in New Jersey, field rots are of relatively small importance; whereas storage rots, caused by fungi which infect the berries in the field, are of paramount importance. Cranberry fungi have been studied intensively by Dr. C. L. Shear and his associates for 25 years. This does not mean, of course, that all cranberry fungi are known. Several unpublished rot fungi have already been found and their descriptions are in manuscript form. Many more will probably be discovered.

The writers have attempted this year to measure quantitatively the actual storage rots caused by the various fungi in cranberries from different growing regions, and to obtain a better idea of the succession of these rots as they develop under ordinary storage conditions. It is hoped to continue the experiment for several years in order to accumulate a fund of information which should prove valuable in future studies along these lines. Because of the amount of work involved, only two varieties could be used in the tests. No given lot can be considered as truly representing the crop in an entire growing section, but in spite of its limitations, in its present stage this is the most complete study of cranberry storage rots ever made, and as such seems to warrant brief presentation.

As in all our work on cranberry diseases, we are indebted to several cranberry growers and others for assistance of various kinds. In this case we are particularly under obligation to the American Cranberry Exchange for furnishing storage facilities in Chicago, and to Dr. H. J. Franklin, who supplied the Massachusetts berries for the experiment.

[1] Investigation conducted cooperatively between the Office of Fruit Diseases, Bureau of Plant Industry, and the Wisconsin Department of Agriculture.

METHODS

Berries were used from four states: Massachusetts, New Jersey, Wisconsin, and Oregon. The last was considered as representing the cranberry region of Washington and Oregon, most of which is located near the mouth of the Columbia River in those two states. One other locality was considered, *viz.*, Long Island, N. Y., but was rejected, as it lies between Massachusetts and New Jersey, which are themselves nearer together than either is to Wisconsin.

The varieties chosen for the tests were the Howes and the McFarlin. The Howes, the standard late variety of Massachusetts and New Jersey, is now being extensively planted in Wisconsin. Although planted in Washington and Oregon early in the development of the industry there, it has not proved particularly satisfactory, and the acreage has been reduced to some extent. The McFarlin is the most important of the so-called fancy varieties. It is the most extensively grown variety in Washington and Oregon, and ranks second only to unselected native vines in Wisconsin. In Massachusetts it is the third variety in importance, but it is grown in only a few places in New Jersey.

The berries were all shipped to Chicago by freight or express without refrigeration. They were shipped in ventilated half-barrel boxes without cleaning or sorting, and were stored together in the shipping boxes until needed for the experiment. About the middle of each month, October to January inclusive, one box of each of the eight lots was opened and a peck of sound berries was sorted out by hand. These eight pecks of sound berries were then stored together in the commercial warehouse of the American Cranberry Exchange in Chicago, under the ordinary storage conditions prevailing in the warehouse, for a period of two weeks. At the end of this period the peck samples were again carefully sorted, and the percentage of berries which had spoiled in the two-weeks period was determined by a numerical count of approximately one-fourth of all the berries in each lot. The spoiled berries were shipped at once to Washington, where cultures were made from 100 rotted berries from each lot or from all the berries if there were less than 100. With the exception of the last two tests, most of the spoiled berries in each sample were cultured; in the last tests, however, too many berries spoiled to permit this. Cultures were made by sterilizing the surface of a berry with mercuric chloride solution and transplanting a portion of the pulp to a culture medium. The tubes were kept in the greenhouse until the fungi fruited or developed sufficiently for identification.

The material for the last three lots of Wisconsin Howes was accidentally lost after it had become impossible to duplicate the samples; consequently the data for these lots are lacking in table 1.

TABLE 1.—*Percentage of loss due to different fungi and other causes in the Howes and McFarlin varieties of cranberries from Massachusetts, New Jersey, Wisconsin and Oregon, in the 1926–27 Chicago storage test*

TABLE 1.—*Percentage of loss due to different fungi and other causes in the Howes and McFarlin varieties of cranberries from Massachusetts, New Jersey, Wisconsin and Oregon, in the 1926–27 Chicago storage test*

Test no.[a]	Sterile breakdown		*Fusicoccum putrefaciens* Shear		*Acanthorhyncus vaccinii* Shear		*Guignardia vaccinii* Shear		*Gloeosporium* sp.		*Ceuthospora lunata* Shear		*Phomopsis* sp.		Total per cent spoiled	
	Howes	McFarlin	Howes	McFarlin	Howes	McFarlin	Howes	McFarlin	Howes	McFarlin	Howes	McFarlin	Howes	McFarlin	Howes	McFarlin
Massachusetts																
1	0.27	0.24	0.09	0.22						0.08			0.42	0.14	1.3	1.0
2	0.48	0.42	0.75	0.36				0.03	0.19	0.11				0.08	1.6	1.0
3	0.53	0.92	0.44	1.70				0.11	0.09	0.65					1.1	4.6
4	2.82	2.50	3.23	1.66			0.13	0.24		0.05			0.53	0.49	6.7	4.9
5	3.90	3.57	3.98	4.04				0.16							8.3	8.1
New Jersey																
1	0.54	0.60	0.49	0.20	0.49	2.60	0.27	1.00	0.05				0.15	0.60	2.7	10.0
2	0.53	0.43	1.53	0.06	0.19	0.61	1.98	1.22		0.12					4.4	2.9
3	0.77	1.73	2.04	2.90	0.06	0.20	0.12		0.12	0.10					3.2	5.1
4	2.59	3.36	4.00	5.64			0.07	0.39					0.13	0.59	7.4	9.9
5	3.02	5.10	5.30	8.26		0.14								0.28	8.4	13.8
Wisconsin																
1	0.24	0.78	2.64	3.11	0.09	0.05						0.19		0.19		6.4
2	0.68		3.16	3.69				0.07							4.7	5.2
3		0.58		5.00		0.25		0.32						0.07	4.2	3.8
4		3.35		4.90		0.09		0.34								8.6
5		6.72		9.73												16.8
Oregon																
1	1.30	1.26	0.80	0.48		0.09					1.40	0.48	0.60	0.33	5.0	3.0
2	0.90	0.09	1.32	0.73							0.30	0.18	0.08	0.05	2.8	1.0
3	2.13	1.23	0.99	0.01	0.04		0.04						0.53		3.8	1.3
4	2.94	5.50	0.28	1.82		0.08		0.23							3.5	7.9
5	13.75	10.55	1.37	2.00		0.43	0.15	0.86			0.15			0.43	15.4	14.3

[a] The five lots under each heading represent the amount of rot developing in samples of sound cranberries which were sorted by hand at the beginning date of each test period and stored as follows: 1.—Oct. 15, 1926. Not stored. Condition of berries upon arrival in Chicago. 2.—Stored from Oct. 15 to Nov. 3, 1926. 3.—Stored from Nov. 15 to Dec. 1, 1926. 4.—Stored from Dec. 15, 1926, to Jan. 3, 1927. 5.—Stored from Jan. 17 to Feb. 4, 1927.

RESULTS

The results are given in table 1, which of course does not include numerous fungi which occurred only a few times. The figures presented were calculated in the following manner: first, the percentages of fungi and sterile tubes from a given lot of berries were determined in relation to the total number of spoiled berries of that lot from which cultures were made. The resulting set of figures of course also showed the corresponding ratios of spoiled berries which had been rotted by the fungi indicated. The figures thus obtained were then multiplied by the percentage of total spoilage or decay that had developed in the stored lot of berries, the latter value having been determined when the sample was sorted. This product, given in table 1, thus represents, within the limits of error under the conditions of the test, the actual amount of destruction in each lot of berries caused by each fungus. The results are shown graphically in figures 1 and 2.

The method of calculation used above is open to some theoretical objections, but it does bring the results from the various localities together on a comparable basis, and shows with some degree of accuracy the approximate amount of loss caused by the various fungi in the test samples. The small size of many of the figures will be readily understood when it is remembered that the total spoilage in the brief period of two weeks is usually not large, and that this spoilage is due to several different organisms as well as to other causes.

The first series of results, number 1 under each lot in table 1, represents the condition of the berries on October 15, a few days after they reached Chicago. The rotten berries present on this date of course include any which may have been rotten when harvested together with those which developed rot in transit. Since the environment of the different lots was not uniform during this time, the results in the first series are not entirely comparable to those of the other four series.

The most striking fact brought out in the table is the preponderant importance of end rot, caused by *Fusicoccum putrefaciens* Shear, in berries of both varieties from all four regions. End rot is by far the most important of storage rots of cranberries from any region, at least under the conditions of our 1926 storage tests. Its importance increased markedly in the later tests, while most of the other fungi tended to decline with the advance of the season. It should be pointed out, however, that the storage temperatures later in the season were much more favorable to the development of the end rot organism than to that of most of the other fungi.

Guignardia vaccinii Shear and *Phomopsis* sp. appeared to some extent in berries from all four regions, although *Guignardia* is of course much more abundant in New Jersey berries than in those from any other state. *Acanthorhyncus vaccinii* Shear, the second or third most important rot fungus

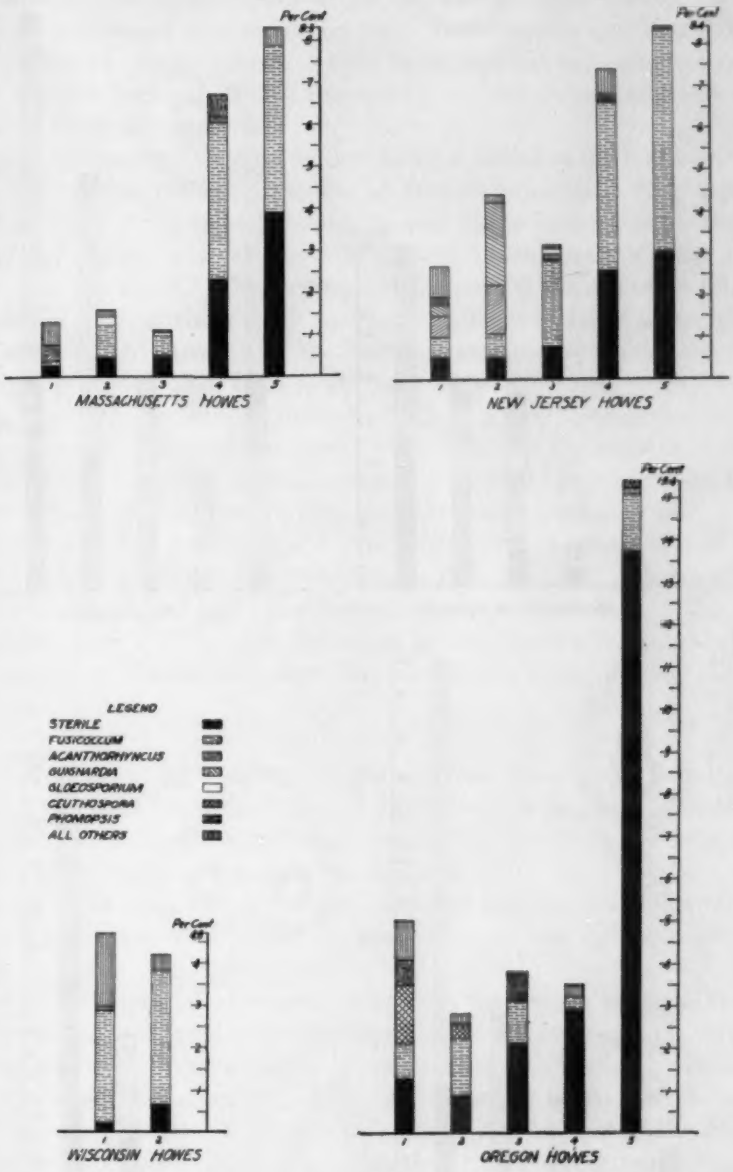


FIG. 1. Percentage of loss due to different causes in the 1926–27 Chicago cranberry storage tests. Columns 1, 2, 3, 4, and 5 in each graph represent the spoilage occurring prior to October 15, between Oct. 15 and Nov. 3, between Nov. 15 and Dec. 3, between Dec. 15 and Jan. 3, and between Jan. 17 and Feb. 4, respectively.

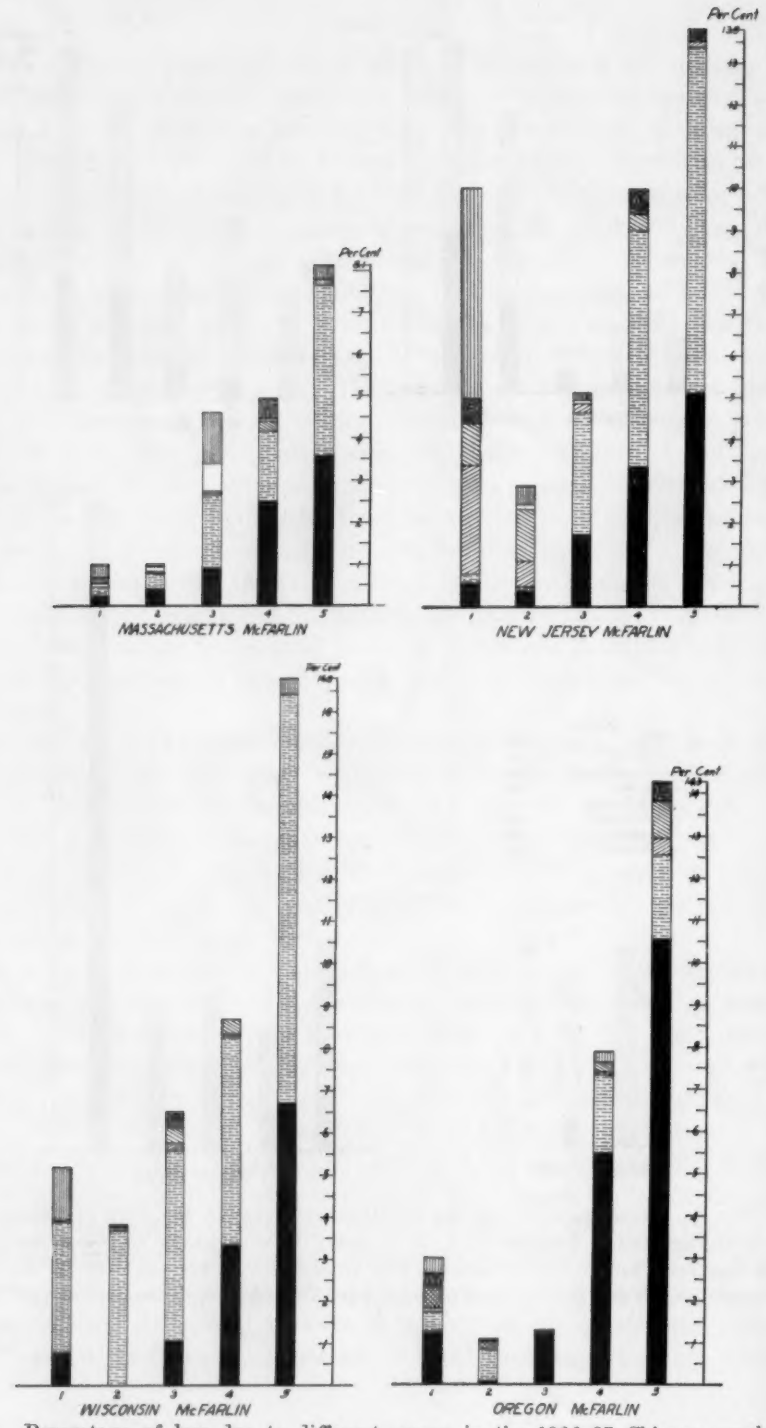


FIG. 2. Percentage of loss due to different causes in the 1926–27 Chicago cranberry storage tests. See figure 1 for legend.

in New Jersey, failed to appear at all in Massachusetts berries. *Gloeosporium* sp. was found only in berries from Massachusetts and New Jersey; and *Ceuthospora lunata* Shear, which is of considerable importance in Oregon, was not found at all in Massachusetts or New Jersey, and only once or twice in Wisconsin material.

Perhaps the most interesting feature of all is found in the first column—the spoiled berries without evidence of fungus infection. This type of breakdown showed the same tendency as end rot to increase with the advance of the season, and was usually present in an amount equal to or greater than the latter. The writers are inclined to consider the relative abundance of berries which spoiled without evidence of fungi as an indication of the inherent strength of the berries. It seems probable that these sterile berries either died what might be termed a natural death, that is of old age, or that they were smothered. In either case the relative number of spoiled berries which proved sterile would indicate the relative strength of the berries of that lot. In this connection it should be noted that, while all the berries were held under identical conditions beginning only a few weeks after harvest, there was a distinct variation, characteristic to both varieties, in the amount of the breakdown which appeared in berries from the different localities. If we are correct in our interpretation of this type of spoilage, there is a decided difference in the inherent strength of the same varieties of cranberries when they are grown under diverse climatic conditions.

SUMMARY

End rot, caused by *Fusicoccum putrefaciens* Shear, was found to be decidedly the most important storage rot fungus in samples of both the Howes and the McFarlin varieties of cranberries grown in the four principal cranberry regions of the United States in 1926.

Several other cranberry rot fungi, together causing a smaller amount of rot than *Fusicoccum*, varied in abundance in the different growing regions.

End rot developed in increasing quantity as the storage season advanced. All other storage rot fungi either decreased in importance with the advance of the season or occurred in about the same degree throughout the season.

A progressively increasing percentage of berries in all samples tested spoiled without evidence of fungous infection. The amount of this type of spoilage varied with the locality in which the berries were grown. Spoilage of this type is believed to be an indication of the inherent strength of the berries as grown under the prevailing climatic conditions.

BUREAU OF PLANT INDUSTRY,
UNITED STATES DEPARTMENT OF AGRICULTURE,
AND
WISCONSIN DEPARTMENT OF AGRICULTURE.

UNIV. OF MICH.

SAND BURN OF PECAN SEEDLINGS

J. B. DEMAREE

Sand burn, a disease of pecan seedlings, was observed by the writer for the first time in a nursery near Cairo, Georgia, during the summer of 1922. Undoubtedly the disease had been present in southern pecan nurseries for several previous years. Mr. O. M. Hadley, Junior Meteorologist, United States Weather Bureau, Thomasville, Ga., informed the writer that he had observed this type of injury in a small nursery in 1912. Since 1922 the writer has seen the disease in nurseries in, or has received specimens from, the following localities: Milledgeville, Shellman, Phlema, Albany, Baconton, Thomasville, and Cairo, Georgia, and Monticello, Florida. The disease was exceptionally prevalent during June and July, 1924.

DESCRIPTION

Sand burn is found in pecan nurseries principally during their first year of growth and for the most part within the period May to July inclusive. The first evidence of the trouble on seedlings that are one to three months old is a small brown spot on the side of the stem which is exposed to the west or the southwest at a point either at or slightly above the soil surface. Following the initial symptom, *i.e.*, the appearance of a brown spot on the side of the stem exposed to the sun's rays, the cortical tissues lying just under the spot collapse and a sunken area results. At the same time the color of the injured surface changes to dark brown or black. Coincident with the breaking down of the cortical tissues lying under the spot, the lesions become somewhat elongated, averaging about one-half inch in length, and frequently extend around the stem, forming a distinct constriction which cuts off the upward flow of sap. The portion of the stem above the lesion dies as a result of complete girdling (Plate XXIV, A). The girdling process appears to extend over a period of several days; consequently the aerial portion of the seedling dies gradually. Injured plants may be detected by certain symptoms several days before they die. The leaves of a severely injured seedling first take on a reddish cast, and about the same time the edges of the leaves roll upward toward the mid-vein. Later the leaves may assume various positions in reference to the axis of the stem (Plate XXIV, B). Some point downward, others stand out at right angles to the stem, and still others retain their normal position, pointing upward and outward. When the lesion completely girdles the seedling, the leaves die

and turn black but do not separate from the stalk. Fragments of the leaves, especially the petioles and rachises, often adhere to the dead stems several months after death. Sometimes the lesions do not extend entirely around the stem, there being a narrow strip of uninjured cortical tissue. An injury of this type usually results in the formation of a callus or swelling immediately above the lesion. Seedlings of this kind may recover entirely and make fairly healthy trees, but more frequently, especially those that have been almost completely girdled, break off during the course of the season. The portion of the seedling below the girdled area does not die as a direct cause of the original injury but sends out sprouts at points on the stalk from one-half to one and one-half inches below the lesion. The fate of such secondary sprouts seems to be determined largely by weather conditions at the time of their emergence from the soil or shortly afterwards. Provided the soil is moist and the air temperature is not excessively high, they usually grow and live through the stage during which they would be susceptible to heat injury. However, these secondary sprouts frequently die when their emergence from the soil is followed by an excessively hot period.

A second form or manifestation of sand burn is that the terminal buds of young plants are killed as they attempt to push through the hot surface layer of soil. This type is more likely to occur in nurseries where the nuts were planted late and the young seedlings emerge in May or June. As many of the injured plants fail to appear, the thinness of the stand, where the injury is severe, is often attributed by growers to failure of the nuts to germinate. As in the case of the girdle type of injury, the portion of the seedling below the point of injury will send up, in some cases, five to seven shoots, simultaneously or successively, which die as they reach the hot surface. Occasionally, one of these shoots manages to grow above the surface, uninjured, but it makes a weak plant of little or no value for propagating purposes (Plate XXIV, C).

FACTORS WHICH SEEM TO BE RESPONSIBLE FOR SAND BURN

Although no experiment has been conducted in an endeavor to produce the symptoms of sand burn artificially, field evidence and laboratory examinations of the lesions indicate that the causative factor is of non-parasitic nature. In general appearance the lesions resemble damping-off diseases attributed to species of *Pythium*, *Corticium*, or *Fusarium*. However, cultures from 169 affected seedlings made during July and August, 1924, failed to develop any damping-off fungi, or any other recognized plant parasite. Most of the cultures developed *Mucor*. Possibly parasitic fungi take some part in the girdling process after the plant tissues have been injured. The fact that the injury usually first manifests itself on the south

or west side of seedlings and during a period of high atmospheric temperatures suggests that high temperature is the predisposing factor. In support of the high-temperature theory, it should be recorded that the most extreme cases of sand burn in pecan nurseries have occurred either on westerly slopes or on dark-colored soil. The highest mortality of pecan seedlings observed by the writer occurred during June, 1924, when the mean maximum shade temperature for the month was 90.7° F. There were 18 days during this month when the temperature was 90° F. and above. During a continuous period of 13 days in this month the daily maximum shade temperatures ranged from 90 to 98° F.[1]

The temperature of the surface layer of dry, sandy soil of the Coastal Plain Region becomes extremely high on hot clear days. One test recorded the temperature of the air one inch above the soil at 119° F., and one inch below the surface at 113° F., but when the thermometer was placed directly on the surface it registered 126° F.

There seems to be an intimate relationship between grade or quality of nuts planted and severity of sand burn. The writer is indebted to Mr. J. L. Pelham, in charge of the United States Pecan Experiment Station, Phlema, Ga., for valuable information on this question. The Phlema nursery was planted in February, 1924, with seed which had been graded into three classes according to size. In May following the planting of the nuts the weather was exceedingly dry and hot, and heavy mortality of the seedlings resulted from sand burn.

The writer visited this nursery on June 6 and again July 17, 1924. While many seedlings were dead and others dying in all plots, there was a marked difference between the stand in the plots planted with high grade nuts and those planted with low grade. Invariably, nuts graded as No. 1 produced more vigorous plants with a smaller percentage of sand burn. A poor stand resulted from planting the lower grade of nuts, owing not so much to low percentage of germination as to death of the terminal buds, either as the plants pushed through the soil surface or became girdled after they had attained a height of from four to six inches.

Sand burn seems also to be associated with late planting. Ordinarily pecan nuts are planted in November and December. Sometimes on account of labor troubles, unfavorable weather conditions, or difficulty of procuring seed, nurserymen delay planting until February or even March. Early planted nuts germinate during March and April, and grow vigorously during the spring months, so that by the time the hot dry weather of summer approaches the young seedlings seem to have grown beyond the stage of susceptibility to heat injury. On the other hand, if the nuts are planted

[1] Climatological Data, United States Weather Bureau Station, Thomasville, Ga.

during late winter or early spring, the seedlings may not appear above the surface of the soil before April or May and consequently are exceedingly tender and susceptible to sand burn during the normally hot dry weather of May and the first half of June.

Sand burn is frequently the most important contributing factor to poor stands of seedlings in pecan nurseries. A deficiency of soil moisture, especially in soils where the sand is from two to several feet deep, may delay the germination of early planted nuts until late spring or early summer. Should the soil surface be excessively hot during the period in which the tips of the seedlings are approaching the soil surface, many would be killed before or during emergence.

ECONOMIC IMPORTANCE

The loss of young pecan seedlings caused by sand burn varies from year to year, and also varies greatly during the same year in different localities. The loss is frequently of very little importance in nurseries planted on level soil of light or moderate fertility and water-holding capacity. During seasons in which high atmospheric temperatures occur during May and June the loss may amount to 50 or 75 per cent on deep, sandy soil, or on hillsides with a western exposure.

HEAT INJURY TO OTHER PLANTS

Hartley[2] reported serious injury to coniferous seedlings which he attributed to excessive temperatures. He recorded a temperature of 126° F. in the surface layer of soil in coniferous seed beds even under half shade of lath frames. Münch[3] described an injury to tree seedlings similar to sand burn of pecan seedlings, and attributed it definitely to excessively high temperature at the soil surface. Other investigators, both in this country and in Europe, have reported stem girdle and other forms of injury to seedlings of trees and herbaceous plants.

PREVENTIVE MEASURES

The planting of high grade seed during early winter, the avoidance of deep sandy soils which are subject to overheating, and the selection of a nursery site not having a westerly exposure greatly lessen the danger of heavy loss by sand burn in pecan nurseries.

[2] Hartley, Carl. Stem lesions caused by excessive heat. Jour. Agr. Res. **14**: 595–604. 1917.

[3] Münch, E. Hitzeschäden an Waldpflanzen. Naturw. Ztschr. Forst. Landw. **11**: 557–562. 1913.

SUMMARY

1. Sand burn of pecan seedlings, which has occurred in southern pecan nurseries for several years at least, is herein reported for the first time.

2. Seedlings in pecan nurseries develop the trouble during the first year of their growth. There are two well-defined types of sand burn. The most striking type is a girdling of the stems at a point near the surface of the soil, which results in the death of the seedlings. The leaves of the dead seedlings turn black but do not fall off at once. In the second type of injury the buds of the young seedlings die as they reach the hot surface layer of the soil. This type of injury, though causing greater mortality of seedlings, is less conspicuous than the first type described.

3. Excessively high temperature at the soil surface is believed to be the direct cause of sand burn of seedlings, but inferior seed and late planting are contributing factors.

UNITED STATES DEPARTMENT OF AGRICULTURE,
THOMASVILLE, GEORGIA.

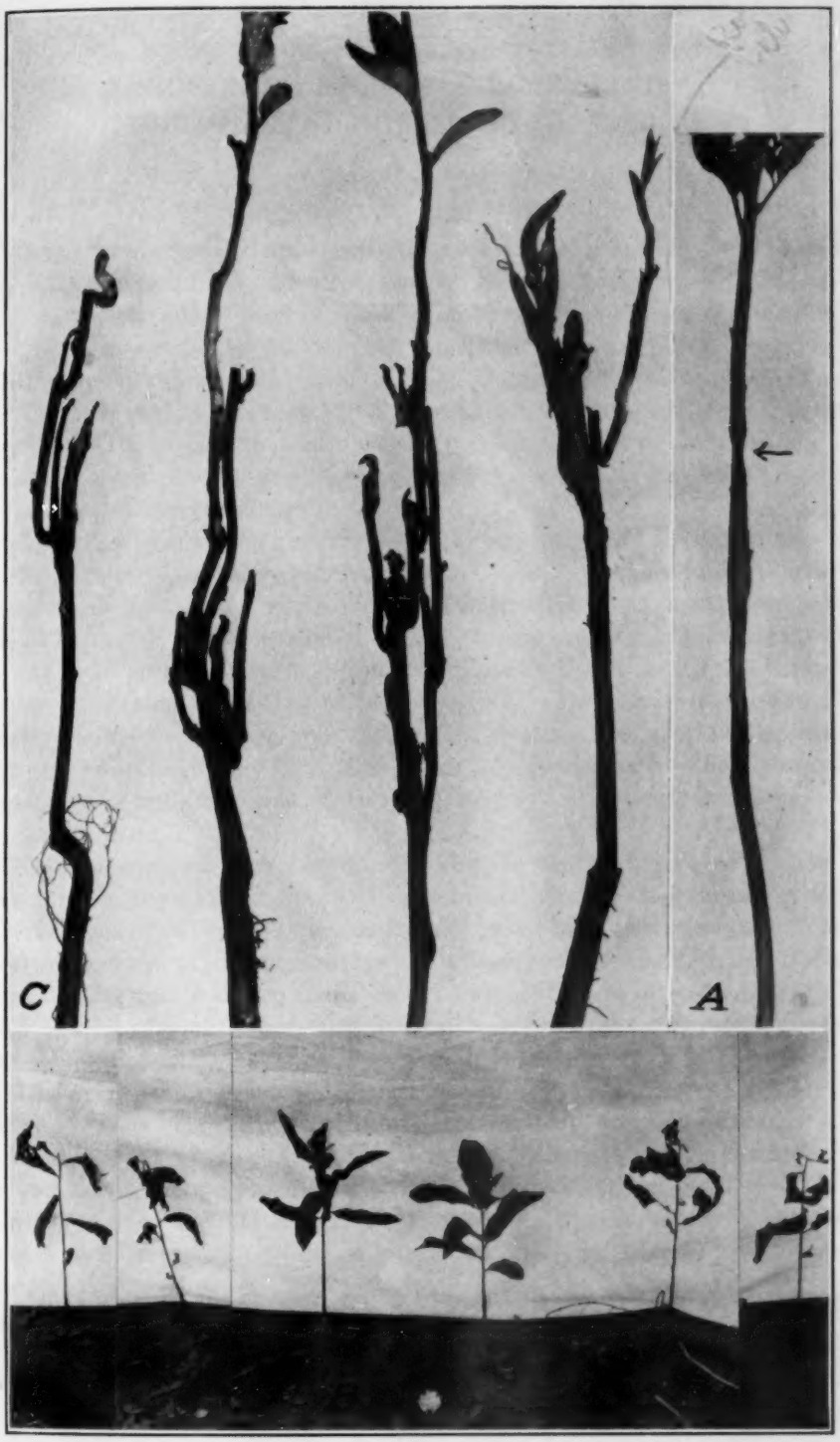
DESCRIPTION OF PLATE XXIV

A.—A two-months-old pecan seedling completely girdled near the soil surface by sand burn injury. Constriction at point of arrow.

B.—Counting from the left, the first, second, fifth, and sixth seedlings have been killed by high soil temperature. The third was dying. Note the distortion of the leaves and their position in relation to the axis of the stem. The fourth plant was uninjured.

C.—Pecan seedlings injured by hot surface soil. The original growing point was killed, whereupon several secondary sprouts developed and in most cases were also killed at the apex by excessive heat.

UNIV. OF MICH.


C
A

UNIV. OF MICH.

THE RELATION OF INSECTS AND WEATHER TO THE DEVELOPMENT OF HEART ROT OF CELERY[1]

J. G. Leach

A heart rot of celery, apparently the same as that described by Wormald (4, 5) and caused by *Bacillus carotovorus,* has been prevalent and destructive in the celery bogs near St. Paul, Minnesota. The disease is characterized by a brown, mushy decay of the small heart leaves. When conditions are favorable, the growing point is killed within two or three days. The stem then begins to lengthen rapidly, and the plant becomes commercially worthless (Fig. 1). Frequently as many as 50 per cent of the plants in a field may be so affected, and on one occasion the writer saw a field that was a total loss due to the disease.

The experience of the growers, and the observations of the writer over a period of four years, show that weather conditions have a profound influence on the development of heart rot. This influence, however, is not what one would expect of a disease caused by *Bacillus carotovorus.* This organism is known to be especially susceptible to desiccation and, as a rule, is most destructive under conditions of high humidity. Destructive outbreaks of heart rot, however, occur only in hot dry weather and are usually most destructive on the drier bogs. Even after the disease has become prevalent throughout a field, a period of rainy weather will apparently check its development completely.

In 1923, investigations were started with the view of finding an explanation for this apparent anomaly. Frequent and minute observations of the development of the disease were made throughout the growing season. The first indications of the disease are the appearance of small, brown spots near the tips and margins of the partly unfolded heart leaves. A careful examination of such leaves with a hand lens has always revealed one or more small dipterous larvae actively working in or about the spots. As the disease progresses, the larvae increase in size, but, on account of the mass of decayed material present, they are evident only on close examination. A large number of the larvae were reared to maturity, and representative specimens of the resulting adults were kindly identified by Dr. J. M. Aldrich of the National Museum. Several species were obtained, but the most prevalent were *Scaptomyza graminum* Fall. and *Elachiptera costata*

[1] Published with the approval of the Director as Paper No. 714 of the Journal Series of the Minnesota Agricultural Experiment Station.

Leow. Both of these species have been reported as leaf miners of cultivated plants. The following account of *Scaptomyza graminum* is taken from Frost (3, p. 93).

"*Scaptomyza graminum* is a European leaf-mining species which has been mentioned many times in American literature. Howard (1896) reports it as occurring in decayed and fermenting fruit. Other writers speak of it as a leaf-miner. Sturtevant (1916) established beyond a doubt the dual habit of the species. Its operations are marked by a large, shapeless blotch, with smaller winding galleries conducting to it.

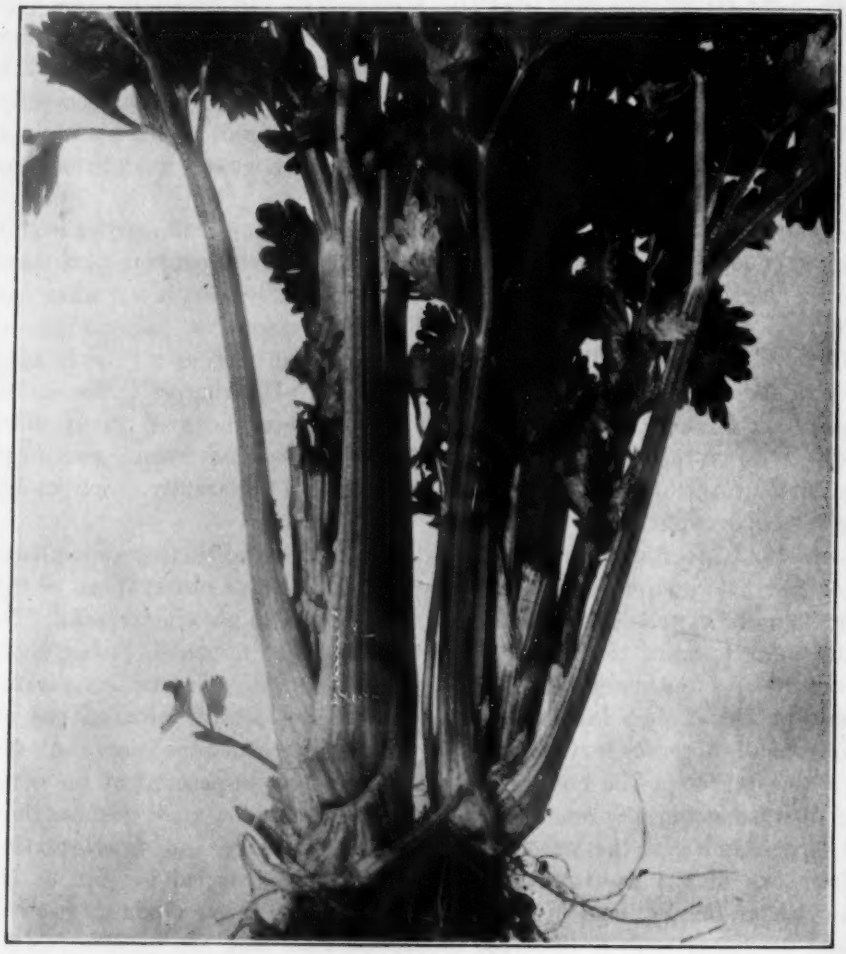


FIG. 1. Two celery plants affected with heart rot. The outer leaves have been removed, exposing the central stalk. This stalk has greatly elongated owing to the destruction of the growing point by the soft rot. Such plants are commercially worthless.

The species has a long list of host plants. . . .

Scaptomyza graminum has been collected in England, France, Germany, and Denmark, as well as in North America. In America it ranges from Canada southward to Georgia, and from the Atlantic coast westward to Minnesota."

Elachiptera costata also has been reported as attacking radish and melon roots (1).

In watching the early stages of development of the disease, small white eggs (Fig. 2) were observed on neighboring healthy leaves and on the heart leaves of yet healthy plants. Some of these healthy leaves which were bearing eggs were cut and placed on moist filter paper in a petri dish. Similar leaves bearing no eggs were cut and placed under similar conditions. The eggs hatched during the following 18 hours, and when the leaves were next examined the maggots were found about one inch from the egg shells, burrowing into the leaves. A small brown discoloration was already evident. This rapidly enlarged, and after five days the leaves were almost completely decayed by typical soft rot. The control leaves remained fresh and turgid (Fig. 2).

Typical cultures of *Bacillus carotovorus* were isolated from the decayed leaves. The larvae were reared to maturity and identified as *Scaptomyza graminum*. The experiment was repeated later, using eggs of *Elachiptera costata* with the same results.

Shortly after an outbreak of the disease in the field in 1923, there occurred a period of prolonged rainfall. Heart rot disappeared from the field and the growers suffered no more losses. Soft rot, however, was found to be just as prevalent as ever, but during rainy weather it was confined to the older outer leaves and petioles. Since these are normally discarded by the grower at harvest, no appreciable loss is felt.

These observations show, then, that the influence of the weather is not so much on the prevalence of soft rot as on the portion of the plant that is attacked. When the heart leaves are attacked and the terminal bud is destroyed, the plant is usually a total loss, while many of the old outer leaves may be destroyed without appreciable loss. A study of the habits of the insect shows why the disease affects the heart leaves in hot, dry weather only. Eggs of the insect are normally deposited in places where the relative humidity is high. In hot, dry weather nearly all of the eggs are found on the younger leaves near the heart of the celery plant. None are to be found on the older, outer leaves. In rainy weather they are found on the outer leaves as frequently as on the inner leaves. Furthermore, when the larvae emerge from the egg, they immediately search for a moist place. In the hottest and driest weather the small heart leaves are always

moist, while the outer leaves are dry. The young larvae, therefore, in dry weather soon find their way to the heart leaves, while in rainy weather they may develop equally as well on the outer leaves. It is also possible that in rainy weather the heart leaves are somewhat more resistant to the decay,

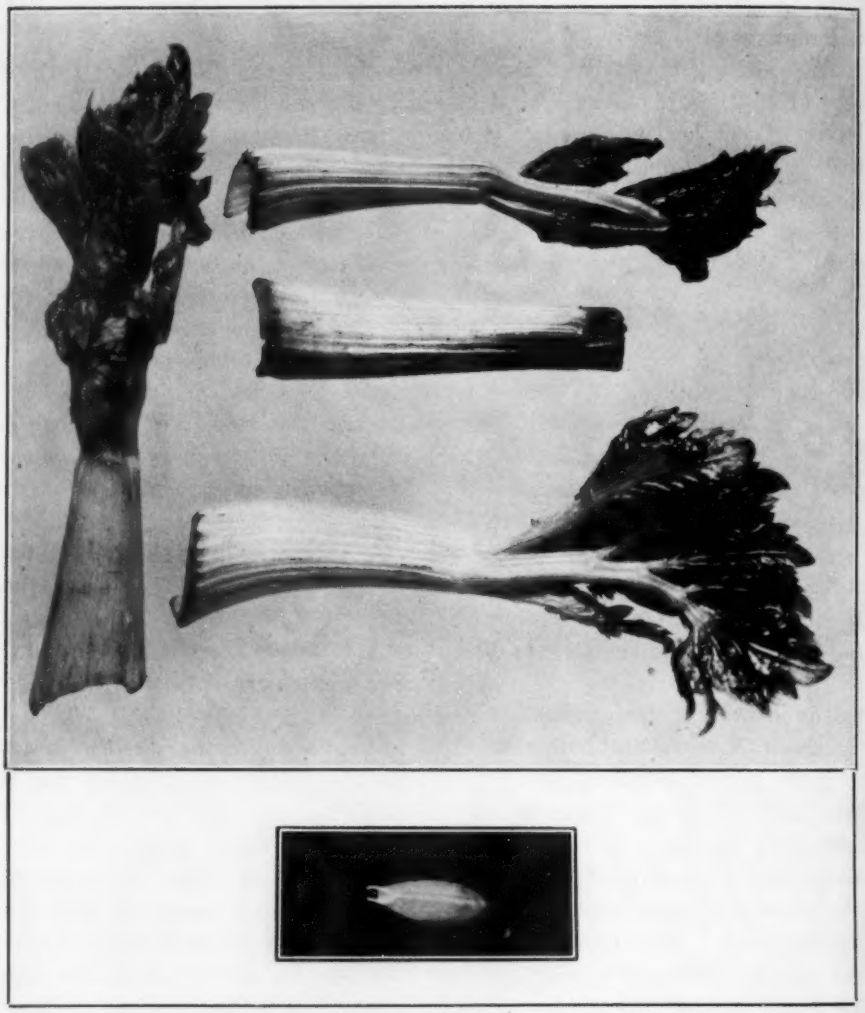


FIG. 2. Young leaves and stems removed from a healthy celery plant and kept in a moist chamber for three days. Those on the right bore one or more eggs of *Scaptomyza graminum*, while the leaf on the left was free of eggs. The decay of the leaves on the left developed as a result of inoculation with bacteria by the larvae which hatched from the eggs and burrowed into the leaves. The insert is a photograph of an egg of *Scaptomyza graminum*, approximately 28 ×.

because heart leaves showing incipient infection have been observed to outgrow the decay.

It was thought at one time that heart rot might be a secondary condition following the non-parasitic trouble known as black heart. This, however, is not the case. Foster and Weber (2), in describing black heart as it occurs in Florida, state: "Blackheart, as found in the South, is distinctly different from 'heart rot' or 'crown rot' which have been reported from northern states and Europe. The latter is a typical soft rot, evidently caused by bacteria of the *Bacillus carotovorus* group, and the former or Florida blackheart is not a soft rot but a typical dry rot followed by premature death of the growing crown." Nothing comparable to black heart has ever been observed by the writer in Minnesota.

SUMMARY

The experiments cited above show that *Scaptomyza graminum* and *Elachiptera costata* are common agents of inoculation of celery heart rot. It is possible that other similar insects may also be equally effective, but it has been definitely proved for these two species only. In addition, these insects in all probability are the chief agents of dissemination, as the adult flies commonly feed on the decaying tissues and have every opportunity to carry viable bacteria from diseased plants to the healthy leaves on which they deposit their eggs.

During the summer of 1926 attempts were made to control the disease by dusting the heart leaves with an insecticide, but shortly after the plants were dusted a prolonged period of rainy weather occurred and the disease did not develop even on the undusted rows. Therefore no results were obtained.

UNIVERSITY FARM,
ST. PAUL, MINN.

LITERATURE CITED

1. COQUILLETT, D. W. On the habits of the Oscinidae and Agromyzidae, reared in the U. S. Dept. of Agr. U. S. Dept. Agr. Bul. Div. Ent. 10 n. s. 1898.
2. FOSTER, A. C., and G. F. WEBER. Celery diseases in Florida. Florida Agr. Exp. Sta. Bul. 173. 1924.
3. FROST, S. W. A study of the leaf-mining Diptera of North America. N. Y. (Cornell) Agr. Exp. Sta. Memoir 78. 1924.
4. WORMALD, H. A bacterial rot of celery. Jour. Agr. Sci. 6: 203–219. 1914.
5. ————. The celery rot bacillus. Jour. Agr. Sci. 8: 216–245. 1917.

UNIV. OF MICH.

NOTES ON THE CERCOSPORELLA LEAFSPOT OF CHINESE CABBAGE IN MASSACHUSETTS

W. H. Davis

The Cercosporella leafspot was first observed on the leaves of Chinese cabbage during September, 1925, and was collected from outdoor plats during each week until January, when the plants were covered with snow. The disease reappeared in the fall of 1926 but was less prevalent and injurious to the crop.

The injury was most severe during the cool weather in autumn: some plants were entirely killed, although others were only slightly affected. The injury was not severe during the main growing and harvesting season.

The initial symptoms appeared in two distinct forms: (a) as minute white, or dilute brown, circular, papery lesions both on young and old leaves —these lesions were definitely delimited, and often dead, dark brown veinlets crossed their surfaces; (b) as minute, yellow, circular spots which spread until the centers were papery, white areas crossed by the dark brown leaf veinlets (Plate XXV, B, 3–4). Fully developed lesions on living leaves were circular and somewhat delimited by the large veins which branched from the midrib (B, 1–2). These lesions averaged 8 mm. in diameter but sometimes coalesced, in which case they appeared as large, dried, irregular, dead areas (A, 5). The margins of the lesions were generally bordered by a dark green area which was seldom raised but appeared more conspicuous in dried herbarium material. White, circular, papery lesions crossed by a network of dark brown veinlets were unmistakable symptoms of this disease.

In the lesions there were two kinds of mycelium: (a) small hyphae, 2–4 microns in diameter, which were mostly intercellular and located near the margins, and (b) hyphae with large cells, 5–6 × 7–34 microns, generally vacuolate and bearing large nuclei. These hyphae were located in the dead, papery, white portion of the lesion.

Sclerotia-like bodies consisting of entwining masses of mycelium formed beneath the epidermis of the leaf. Very short and sometimes indistinguishable conidiophores originated from these mycelial masses. Structures which appeared like conidiophores were unbranched, mostly cespitose but sometimes single; mostly erect but sometimes detached and prostrate on the surface of the leaf; hyaline, averaged 3 microns in diameter and varied from 0 (indeterminate) to 13 microns in length. It was not decided whether these were conidiophores, immature conidia, or cells of broken conidia which remained attached to the sclerotia-like bodies. However, some conidia origi-

nated from hyphal strands which had emerged from the leaf tissues by penetrating the epidermis. The conidia were hyaline, 1- to 7-, mostly 3- to 4-celled, elongate-cylindrical, seldom fusiform and constricted at the cross-walls, mostly curved but often straight. Limits for the measurements of 100 conidia: 1.5–3.5 × 22–100 microns; standard, 2.5 × 56 microns.

Beets, celery, parsnips, carrots, and common cabbage were inoculated with conidia from Chinese cabbage to determine the hosts of this fungus (series I). Beets, celery, and parsnips growing in nearby plats were infected with Cercospora species, so reciprocal inoculations were made with conidia from Chinese cabbage and each of the other three hosts (series II).

In series I, five different inoculations were made on each of the plants which were cultured under controlled greenhouse conditions. The Chinese cabbage plants were the only ones in this series that became infected. In series II, three different multiple inoculations were performed. In all inoculations the Chinese cabbage remained healthy unless inoculated with conidia from Chinese cabbage. The *Cercospora* from Chinese cabbage did not infect the other hosts inoculated.

In view of the fact that species of *Cercospora* which infect Chinese cabbage and various other crucifers have not been definitely described and comparatively few inoculations reported at the present time, it is difficult to determine species and races definitely. The negative results from some of the inoculations previously described might have been due to physiological specialization (races).

From the descriptions at hand and the examination of herbal material available, it appears that *Cercospora bloxami* B. and Br., *Cercospora albo-maculans* E. and E., and *Cercosporella brassicae* Jaap. are synonymous with *Cercosporella albo-maculans* E. and Ev. (Saccardo, "Sylloge Fungorum" **11**: 606. 1895).

The genus and species of the organism which causes this disease on Chinese cabbage should be reported as *Cercosporella albo-maculans* (E. and Ev.) Sacc. until further cultural studies and comparisons of organisms from a larger number of hosts present data to the contrary.

DEPARTMENT OF BOTANY,
MASSACHUSETTS AGRICULTURAL COLLEGE,
AMHERST, MASSACHUSETTS.

DESCRIPTION OF PLATE XXV

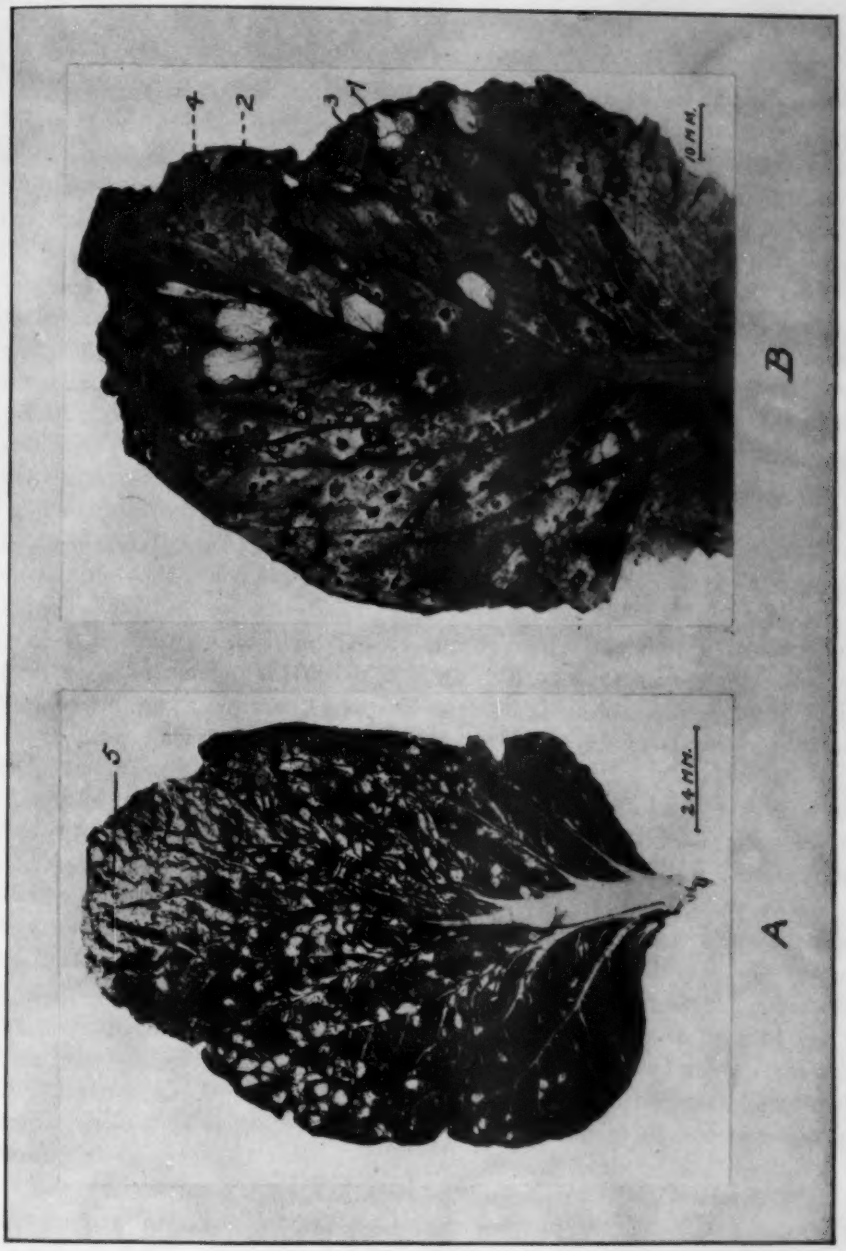
Photographed leaves of Chinese cabbage infected with *Cercosporella albo-maculans*.

A. Numerous lesions which have coalesced at the tip of the leaf (No. 5).

B. A young, infected leaf.

No. 1–2. Large, orbicular lesions of paper-like appearance crossed by the dark brown veinlets. Pressed leaves sometimes retain dark green margins around the lesions (No. 3).

No. 3–4. Early symptoms.


4
2
3
1
10 MM.
B
5
24 MM.
A

UNIV. OF MICH.

PHYTOPATHOLOGICAL NOTES

Preliminary Report on the Gardenia Bud Drop.—One of the greatest trials of nurserymen who grow gardenias in quantity is the so-called "bud drop"—the falling off of buds shortly before flowering time. In the San Francisco Bay region, bud drop caused a loss of $2,000 in one nursery alone in 1925.

The disease first becomes evident as a gradual discoloration of the pedicel and bud. The disease develops on the bud on the exterior of the calyx, where there are five pore-like structures, the extra-floral nectaries. This mode of entry of plant disease through extra-floral nectaries has not hitherto been described. The bright green color of the buds becomes dark cream, then yellow and finally dark brown. The bud gradually becomes moist and rotten and finally drops off in from four days to a month, the time depending on the temperature.

Thinking that the disease might be bacterial in nature, isolations were made on alfalfa agar from buds in various stages of infection. Within three days minute bacterial colonies were visible; two days later these were an inch in diameter, and all alike.

After pure cultures had been secured, many inoculation experiments were made. In one of the series, 20 healthy buds were inoculated. Most of these inoculations were made in the extra-floral nectaries of healthy buds. When leaves and stems were inoculated, the infection developed only when high temperatures prevailed.

In each experiment some of the buds were used as controls, but in every case only a few blossomed. The fact that a few controls did blossom shows that insect control and clean surroundings for the plants are important factors.

Careful observation in commercial greenhouses and under the controlled conditions of the laboratory established the fact that the disease was spread by mealy bugs and ants, which usually infest the beds in which the gardenias are grown. Experiments were repeatedly performed with insects taken from infected beds and with insects obtained from regions remote from any gardenia plants. Both mealy bugs and ants feed at the extra-floral nectaries, hence it is very probable that the disease may become widespread through these agents.

Temperature has a direct influence on the activity of the bacteria: higher temperatures are more favorable for their growth.

The bacteria causing the disease are rod-shaped, occurring singly for the most part, though occasionally two are joined endwise. Each bacterium

possesses approximately seven peritrichiate flagella. The organism therefore belongs to the group *Erwinia*. The colonies are smooth, circular in outline, of a yellowish hyaline mucose appearance, with a milky spot in the center of older colonies.—ALBERT WILSON, *Department of Botany, Stanford University, California.*

The American Type Culture Collection.—The recent publication of the first printed catalogue of the American Type Culture Collection of bacteria and fungi will awaken interest in this effort to preserve authentically-named viable cultures in a central place for distribution. Copies of the catalogue may be obtained from Dr. George H. Weaver, John McCormick Institute, 637 South Wood Street, Chicago. Cultures may be purchased from him at one dollar per culture, plus the cost of packing and postage. There are now about 200 carefully selected pure cultures of fungi in the collection. They are cared for by Mario Scandiffio, who works half time, under the supervision of Drs. Charles Thom and Margaret B. Church, of the United States Department of Agriculture, and is paid by the General Education Board and the Society of American Bacteriologists. Each culture is kept on a suitable medium in three places, representing different combinations of humidity with average temperatures of 0°, 7°, and 24° C. The cultures are transferred at intervals of 3, 6, and 12 months, depending on the organism and the temperature at which it is being maintained. As this arbitrary schedule is supplemented by continual supervision, few cultures have been lost.

Phytopathologists are urged to contribute cultures, both of newly described species and of strains of previously described species which they have studied. Each culture should be accompanied by as complete a history as possible and by references to the original description of the species and to special investigations.—ANONYMOUS.

Fifth International Botanical Congress, Cambridge, 1930.—At the International Congress of Plant Sciences (Fourth International Botanical Congress) held at Ithaca, New York, United States, in August, 1926, an invitation was conveyed from British botanists for the Fifth International Botanical Congress to be held in England in 1930. The invitation was accepted by the botanists assembled at Ithaca, and arrangements are now being made for the Congress to be held at Cambridge about the middle of August, 1930.

An Executive Committee has been formed to make arrangements for the Congress, consisting of Dr. F. F. Blackman, Professor V. H. Blackman, Dr. E. J. Butler, Professor Sir John Farmer, Professor F. E. Fritsch, Professor Dame Helen Gwynne-Vaughan, Dr. A. W. Hill, Professor W. Neilson Jones,

Sir David Prain, Dr. A. B. Rendle (treasurer), Professor A. C. Seward (chairman), Professor W. Stiles, and Professor A. G. Tansley.

It has been decided to organize the Congress in the following seven sections: morphology (including anatomy), palaeobotany, plant geography and ecology, taxonomy and nomenclature, genetics and cytology, physiology, and mycology and plant pathology.

Mr. F. T. Brooks, The Botany School, University of Cambridge, England, and Dr. T. F. Chipp, Royal Botanic Gardens, Kew, England, have been appointed honorary secretaries of the Congress, and any communications with regard to the Congress should be addressed to one or other of the secretaries.

United States Distributors

of

Potash Salts of All Grades

N. V. POTASH EXPORT MY.

of

Amsterdam, Holland

New York Offices

20 West 45th Street

Atlanta, Ga.
Hurt Building

Baltimore, Md.
Citizens' Natl. Bank Building

Plant Pathologists Recommending

BORDEAUX MIXTURE

Should know the Remarkable Efficiency of

SUNOCO SPRAY OIL

as a Spreader and Sticker

One gallon to 200 gallons of Bordeaux. Especially recommended for Grape and Potato Sprays.

Always ready. **Mixes quickly.**

Will not deteriorate.

Write for descriptive booklet

SUN OIL COMPANY

PHILADELPHIA, PA.

PH7

Two Books by Whetzel

PLANT PATHOLOGY

Professor Whetzel, the senior author, has given this book a thorough revision for the second edition. It is intended for a four year course and aims to acquaint the student effectively with the laboratory materials presented.

Laboratory Outlines in Plant Pathology. By H. H. Whetzel, Lex R. Hesler, Charles T. Gregory, W. Howard Rankin. Octavo of 231 pages. Cloth, $3.25 net.

HISTORY OF PHYTOPATHOLOGY

Professor Whetzel divides his work into Eras and Periods. He gives a general survey of each Era and each Period, crystallizes the evolutionary movement of each, gives interesting biographic sketches of the predominant figures, frequently including portraits.

History of Phytopathology. By Herbert Hice Whetzel, Cornell University. Cloth, $2.00 net.

W. B. SAUNDERS COMPANY

Philadelphia **London**